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Osteoporosis

Edited by Yannis Dionyssiotis

OSTEOPOROSIS

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Meet the editor

Dr Yannis Dionyssiotis studied Medicine at the University of Athens, and specialized in Physical Medicine and Rehabilitation in the National Rehabilitation Center EIAA in Athens. He was a Research Fellow in the Laboratory for Research of the Musculoskeletal System at the University of Athens, where he completed his thesis in osteoporosis. From 2005, he has been Fellow of the European Board of Rehabilitation. He worked in the Rehabilitation Department of KAT Hospital in Athens, as Head of the Physical Medicine and Rehabilitation Department in Rhodes General Hospital, and is currently the Medical Director of the Physical and Social Rehabilitation Center Amyntaeo in Florina, Greece. He is an elected member of the Board of the International Society of Musculoskeletal and Neuronal Interactions (ISMNI), has been elected twice onto the Board of Hellenic Osteoporosis Foundation (HELIOS), has written medical books and chapters in the English and Greek language, and many papers in international journals. Additionally, Dr. Yannis Dionyssiotis won the Heim Ring Silver award in October 2010, and the European Board award in June 2008. He is a reviewer in many PubMed journals and also the editor of the online rehabilitation magazine medreha.com.

Contents

Preface

Osteoporosis is a public health issue worldwide. During the last few years, progress has been made concerning the knowledge of the pathophysiological mechanism of the disease. Sophisticated technologies have added important information in bone mineral density measurements and, additionally, geometrical and mechanical properties of bone. New bone indices have been developed from biochemical and hormonal measurements in order to investigate bone metabolism. Although it is clear that drugs are an essential element of the therapy, beyond medication there are other interventions in the management of the disease. Prevention of osteoporosis starts in young ages and continues during aging in order to prevent fractures associated with impaired quality of life, physical decline, mortality, and high cost for the health system. A number of different specialties are holding the scientific knowledge in osteoporosis. For this reason, we have collected papers from scientific departments all over the world for this book. The book includes up-to-date information about basics of bones, epidemiological data, diagnosis and assessment of osteoporosis, secondary osteoporosis, pediatric issues, prevention and treatment strategies, and research papers from osteoporotic fields.

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Part 1

The Basics of Bone

1

Bone Mineral Quality

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1. Introduction

The main function of bone is to promote locomotion and protection of vital organs. Bone is also an important mineral ions reservoir, essential to maintain phosphocalcic homeostasis. Bone mineral is a calcium phosphate named "apatite", which form naturally in the Earth's crust (Wopenka & Pasteris 2005). Compared to others minerals, apatite is more "tolerant" and is very accommodating to chemical substitutions. This ability to easily absorb ions confers to bone a detoxification property, with some ions normally absent of bone and which are captured by bone mineral. But the substitutions in bone mineral change the structure of apatite, conferring to bone several properties such as solubility, morphology, hardness, strain etc.

Thanks to those remarkable properties, bone has the ability to continually adapt to changes to its mechanical environment (Bouxsein 2005). Bone is an anisotropic composite material tissue, and highly hierarchical viscoelastic (Bouxsein 2005). When a load is applied to bone, this produces energy, and as this energy can not be destroy, the bone has to absorbed it (Seeman & Delmas 2006). The elastic properties of bone allow to absorb this energy by deforming reversibly. But if the load exceeds the ability of the bone to carry this load, it can deform permanently by plastic deformation (Fig.1). This produces microcracks allowing

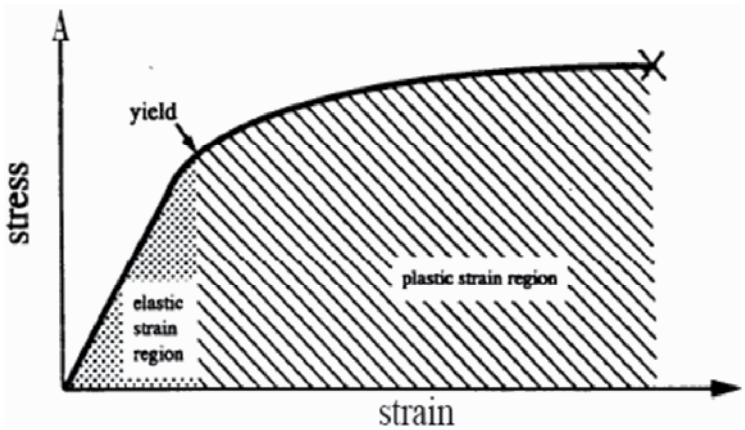

Fig. 1. Stress-strain curve divided into the elastic and plastic regions. The fracture occurs at the end of the curve (marked with **X**). Reprinted from Turner & Burr, 1993, with permission from Elsevier.

energy release. If the microcracks remain small, this has no impact on bone. However, if the microcracks become numerous and/or too long, the bone fractures. Thus, to resist to a fracture, the bone need to find the best compromise between *stiffness* and *flexibility* (to resist deformation) (Seeman & Delmas 2006). A high mineral content increases *stiffness* reducing *flexibility,* and if the bone is too flexible, it will deform beyond its peak strain and crack. Several studies showed that mineral part is involved in the elastic properties of bone, whereas organic part is rather involved in the plastic deformation (Bala et al., 2011b; Bouxsein 2005; Currey 2003; Follet et al., 2004).

2. Determinants of bone quality

Bone is constituted by three major components which are the organic matrix (≈30%, mainly type I collagen), mineral (≈60%, carbonated apatite) and water (≈10%). Organic matrix is essentially constituted by ~ 90% of a network of type I collagen fibrils, and ~10% of non-collagenous proteins. Type I collagen molecules are formed by three polypeptides α chains [2 α1(I) and 1 α2(I)] forming a tight triple helix structure with a repetition of Gly-X-Y triplets (Myllyharju & Kivirikko 2004). To provide the stability of type I collagen fibrils, several mechanisms of maturation and ageing of bone collagen occurs, including enzymatic collagen cross-linking and non-enzymatic modifications (Saito & Marumo 2010; Viguet-Carrin et al., 2006). In parallel, the organic matrix mineralizes, and the organization of type I collagen network determines the specific arrangement of mineral crystals (Höhling et al., 1990; Riggs et al., 1993). The crystals first grow in length, typically plate-like (Landis, 1995; Roschger et al., 1998), then they become thicker but stay relatively thin (Roschger et al., 1998). Concomitantly, the crystals number increases up to physiologic limits of mineralization (Bala et al., 2010; Boivin et al., 2008; Boivin & Meunier 2002).

The quantity of bone mineral (assimilated to the quantity of total bone) is usually measured by bone mineral density (BMD), using dual X-ray absorptiometry (DXA). However, about one-half of fractures occur in women having a T-Score above the World Health Organization (WHO) diagnosis threshold of osteoporosis (≤-2.5) (Siris et al., 2004; Sornay-Rendu et al., 2005) suggesting that other factors than bone quantity are involved in the apparition of fractures. These factors, involved in bone quality, are called intrinsic determinants. Both extrinsic determinants (including bone mass, macro/microarchitecture) and intrinsic determinants are involved in bone strength, and are directly dependent of bone remodeling activity (Fig.2).

DXA measurement gives information on bone mineral mass but not on its mineral quality. For example, when fluoride salts was used to treat post menopausal osteoporosis, an increase in bone mineral density was measured by DXA, but the bones of patients treated with fluoride salts were much more brittle than untreated patients. In fact, fluoride ions in bone mineral impaired the bone mineral quality increasing the size of bone (large crystals), and thus reducing the contact area with collagen matrix, despite a higher amount of bone mineral density.

The determinants of bone quality, called ”intrinsic determinants”, are thus essential in bone strength. Those determinants include mineral quality, collagen quality, and presence of microcracks. A good collagen quality is required to an optimal bone strength. For example, osteogenesis imperfecta, which is an heritable brittle-bone disease, is characterized by a type-I collagen mutation, leading to collagen fibrils abnormally thin, and to an excessive

bone fragility (Rauch & Glorieux 2004). Among these determinants, microcracks are normally present in bone, and permit to dissipate energy when bone is submitted to a load. However, the presence of too long microcracks is not good for bone. Thus, all together those extrinsic and intrinsic determinants are involved in bone strength.

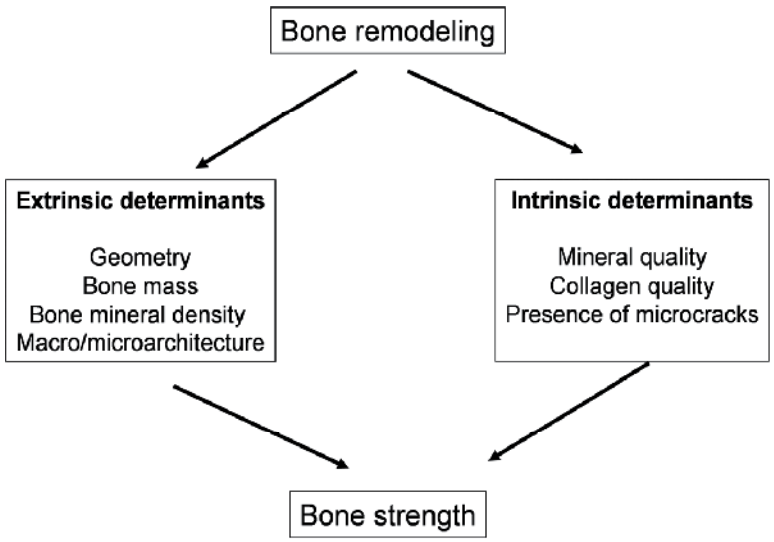

Fig. 2. Description of determinants of bone strength (INSERM UMR 1033)

Bone mineral properties are important determinants of bone strength. Those properties include material (degree of mineralization, hardness…) and crystalline (crystallinity, mineral maturity, ionic substitutions...) characteristics. The importance of the components of bone quality is thus evident and the relationships between the determinants of bone quality are essential to maintain an overall mechanical competent bone. Indeed, bone mineral is complex and knowledge of its composition is important to better understand the mechanisms of bone fragility.
The main purpose of this present chapter is to bring a best approach of bone mineral quality, mineralization process, mineral crystals and mineral composition.

3. Mineralization process: A dynamic process

As bone is submitted to a constant remodeling during all the adult life, old Bone Structural Units (BSUs, named the osteons in cortical bone and the trabecular packets in cancellous bone) will be resorbed by osteoclasts, and replaced by new formed bone. Thus, the recently formed BSUs will be less mineralized than the older BSUs present in interstitial bone and not already resorbed. This heterogeneity of mineralization in the different BSUs can be easily visualized on a X rays microradiograph (Fig. 3). This mineralization process related to bone remodeling can be decomposed into two steps: a primary mineralization which corresponds to a very rapid deposition of first crystals, and a secondary mineralization which is much longer, with a slow and gradual increase in size, perfection and number of crystals.

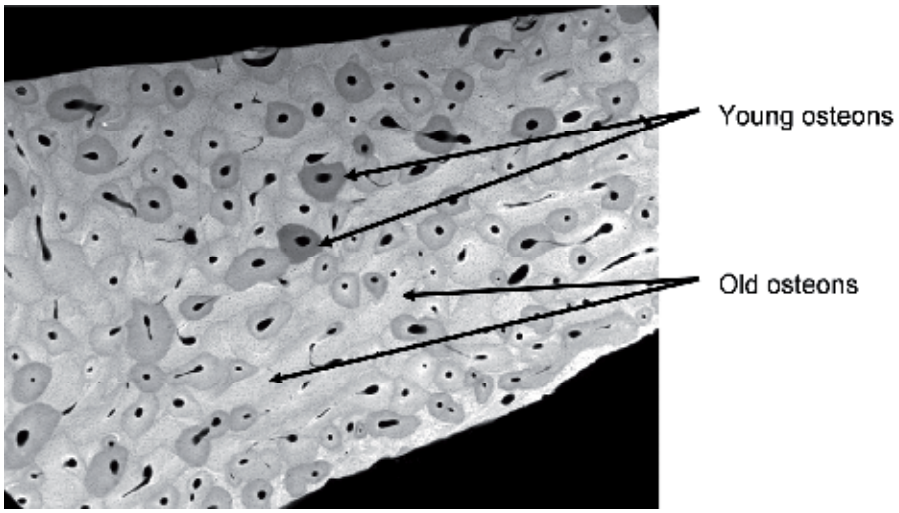

Fig. 3. Microradiograph of human femur illustrating the heterogeneity of the mineralization (cortical bone of a man, 48 year-old) (INSERM UMR 1033)

3.1 Primary and secondary mineralization processes

The process of primary mineralization is a very rapid process, starting in the unmineralized bone matrix (osteoid) deposited by the osteoblasts; in humans, the new matrix begins to mineralize after 5 to 10 days after the deposition of osteoid. The primary mineralization can be measured using double tetracycline labeling (Frost 1969) (Fig. 4). The double labeling involves the administration of two short courses of tetracycline which is deposited along the calcification front as two distinct lines visualized on bone sections under ultraviolet (UV) light. This allows the measurement of the mineral apposition rate (MAR). Usually, a labeling procedure is 10 mg/kg/day demethylchlortetracycline or tetracycline hydrochloride orally for 2 days, 12 days off, 4 days on. The bone biopsy is then taken 4-6 days later (Frost 1969).

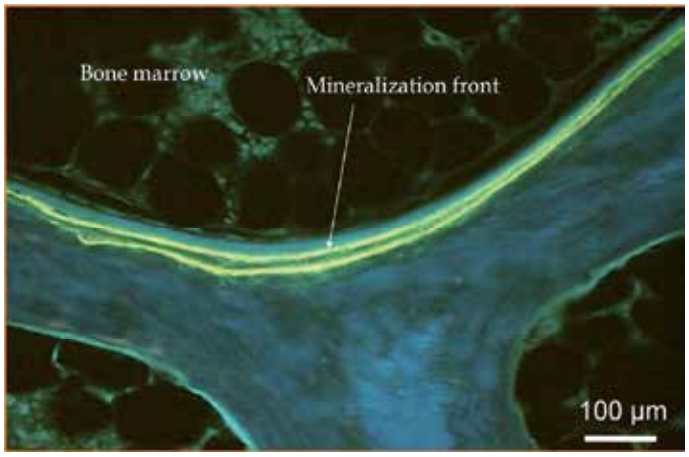

Fig. 4. Histological slide of bone tissue observed under UV light and illustrating a double tetracycline labelling (yellow lines) in trabecular bone and showing the front of mineralization (INSERM UMR 1033).

In adult, MAR varies from 0.60 to 0.80 µm/day whatever the age and the sex (Vedi et al., 1983). MAR is slightly increased in young children, reaching 1 µm/day (Glorieux et al., 2000).
During the process of primary mineralization, the first depositions of mineral correspond to about 50 to 60 % of the maximal mineral charge in bone tissue (Meunier & Boivin 1997). This process is extremely rapid, and the first depositions of mineral are used as nucleator for the secondary mineralization. The secondary mineralization corresponds to a slow and gradual increase in both crystal size and number. This process increases until a physiological limit: once the maximum number of crystals attained in a given volume, it is not possible to exceed this limit. Thus in bone remodeling, there is no process of "hypermineralization", because a given BSU can not contain more crystals than its physiological capacity.
The duration of the secondary mineralization is unknown in humans. This duration has been reported in rabbits (Fuchs et al., 2008) and more recently in an animal model (ewes) having a remodeling activity close to the Humans (Bala et al., 2010). The chronology of secondary mineralization has been identify by injection of different fluorescent labels every six months, in order to date the "age" of the BSUs (Bala et al., 2010). In this study, it has been shown that the secondary mineralization lasts approximatively for 24 to 30 months, suggesting that after this time, no increase in degree of mineralization occurs (Fig. 5).

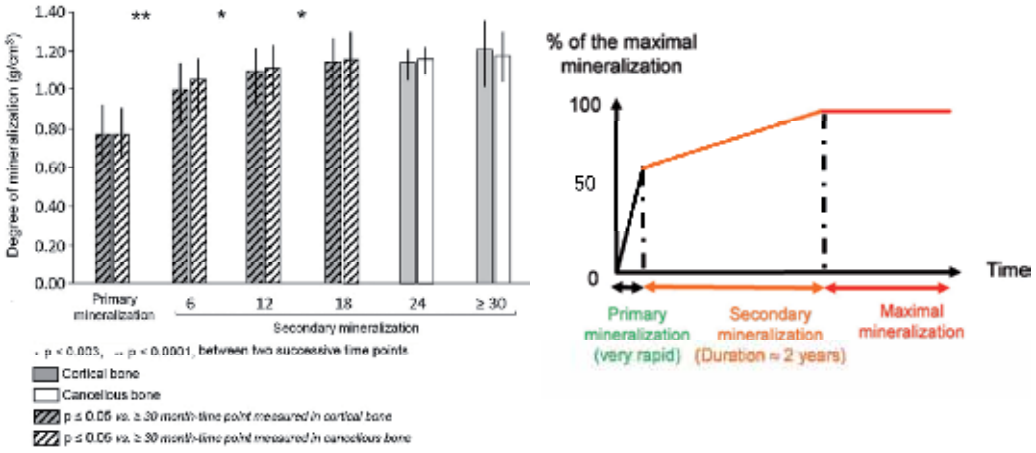

Fig. 5. Left: Degree of mineralization measured by quantitative microradiography in ewes in cortical and cancellous bone, every 6 months (on 512 BSUs; Reprinted from Bala et al., 2010, with permission from Elsevier). Right: Schematic representation of the duration of primary and secondary mineralizations.

This duration of mineralization should be taking into account into anti-resorptive treatment of post menopausal osteoporosis, because once that all the BSUs have attained their maximal mineralization, no gain in term of DMB will occurs.

3.2 Methods to measure degree of mineralization

Several methods are used to measure the degree of mineralization of bone. The technique used in the laboratory is the quantitative microradiography, which is a computerized microdensitometric method based on the X-rays absorption (Boivin & Meunier 2002). Others methods are used to measure the degree of mineralization, as the quantitative backscattering electron or synchrotron infrared microspectroscopy.

3.2.1 Quantitative microradiography

3.2.1.1 Specimen preparation

Undecalcified iliac bone samples were generally used in humans, fixed in 70% alcohol for ten days or more (depending on the size of the samples), and then specimens are placed two days in absolute alcohol to complete dehydration. Alcohol baths are changed every day and specimens are substituted in methylcyclohexane again for two days, before embedding in methyl methacrylate (MMA). The latter is a transparent and hard plastic having a very low X-ray absorption power. Samples are kept for two days in MMA monomer alone, at 4°C, two days in MMA with 1% of catalyst (anhydrous dibenzoyl peroxide) and 2 days in MMA with 2% of catalyst. Then the specimens are placed in an oven (30°C) for final polymerization to obtain hard blocks. After polymerization, thick sections are cut from the embedded bone samples with a precision diamond wire saw, progressively ground to a thickness of 100µm and polished with an alumina suspension. The thickness of the section was measured with an accuracy of 1 µm using a precision micrometer. After ultrasonic cleaning in demineralised water, the bone sections were microradiographed. If orientation of the blocks is possible before sectioning, the cutting plane perpendicular to the haversian canals of cortical bone is preferred.

3.2.1.2 Measurement of degree of mineralization (DMB) (Boivin & Meunier 2002)

Soft X-rays are produced in a X-ray generator (Philips compact PW1830/40 X-ray diffraction generator, Limeil Brévannes, France), equipped with a diffraction tube PW 2273/20. A monochromatic X-ray beam is employed, i.e., nickel-filtered copper Kα radiation with a wavelength of 1.54 Å for which the ratio of the mass- absorption coefficients of aluminium to apatite is 0.561. The distance between the X-ray source and the specimen is about 25-30 cm. In a dark room, the 100 µm-thick bone sections are placed on a photographic emulsion covered by a thin polyester (mylar) film transparent to X-rays, and placed in a specimen holder. An aluminium step-wedge is also exposed on each microradiography. Aluminium was chosen because it is convenient material having an atomic number not far from the effective atomic number of hydroxyapatite. The section is firmly pressed flat by tightening the specimen holder cap and evacuating the air situated between the mylar and the emulsion by means of a vacuum pump, thus bringing the section in direct contact with the emulsion. The specimen holder is placed in a camera perpendicular to the X-ray beam and locked into position during X-ray exposure, during 20 min at 25 kV and 25 mA.
After X-ray exposure, the film (VRP-M green sensitive emulsion from Geola, Slavich International Wholesale Office, Vilnius, Lithuania) is developed for 5 min in Kodak D19 at 20°C, rinsed and then fixed for 5 min in Ilford Hypam. The film is washed and dried, then mounted between two slides. The DMB is quantified using an automatic program for analyzing grayness levels (MorphoExpert and Mineralization, ExploraNova, La Rochelle, France). A digital camera (resolution: 1600 x 1200 pixels or 800 x 600 after binning), captures the microscopic image of the microradiograph. After calibration with the aluminium reference system, the measured regions of bone tissue are automatically selected, and the gray levels are segmented after bone thresholding. The values of the gray levels are then obtained at pixel level (for a magnification x2.5, the size of the pixel is 2.82 µm). Finally, gray-level are converted into DMB measurements with the construction of a calibration curve based on the measurements obtained on the aluminium step-wedge. DMB is finally expressed in gram of mineral over cm^3 of bone (g/cm^3) and measured separately in cortical

and cancellous bone. The main parameters, extracted from the DMB measurements, are the mean DMB, the mean highest and most frequent DMB (DMB Freq. Max) and the mean index of heterogeneity of the distribution of DMB expressed as the mean of the widths at half-maximum measured on the individual DMB curves.

3.2.2 Quantitative backscattering electron imaging (qBEI)

The mineral content of bone samples has also been evaluated (Roschger et al., 1995, 1998, 2003; Ruffoni et al. 2007) by quantitative Backscattered Electron Imaging (qBEI). This method, based on the detection of electrons backscattered (BSE) on the surface of the bone specimen, is generally used on the same type of bone biopsy fixed in alcohol and embedded in MMA. As the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images provide information about the distribution of different elements in the sample. A calibration of the BE signal with carbon and aluminium as references was performed. Osteoid and hydroxyapatite were also employed as references to convert gray level values into calcium weight % values. In bone, the main signal is related to Ca (Z=20) and P (Z=15) which are the main mineral elements (Roschger et al., 1998). These authors have correlated BE gray levels of bone with calcium content (in weight percent Ca) based on the Ca Kα-line intensities detected from identical bone areas (Roschger et al., 1995). The intensity of the backscattered electron signal from the sample is directly proportional to the bone calcium concentration and can therefore be used for the generation of bone mineralization density distribution (BMDD). BMDDs display the frequency of certain calcium concentrations and are analyzed for the weighted mean calcium concentration (Ca mean), the most frequent calcium concentration (Ca peak) and the homogeneity of mineralization (Ca width). The BMDD of trabecular bone from healthy, adult individuals was shown to be nearly constant over several biological factors (gender, age, ethnicity, skeletal site). Technical and biological variations showed that it is a method sensitive for subtle changes in mineralization.

3.2.3 Synchrotron radiation microtomography (SRμCT)

Aside from the difficulty of access to synchrotron radiation facilities, a main advantage of this technique is the use of a mono-energetic synchrotron beam, thus avoiding beam-hardening effects. Indeed, the reconstructed gray levels of tomographic images correspond directly to a map of a linear attenuation coefficient within the sample. The SRμCT method has been tested on human bone tissue (Borah et al., 2005, 2006; Nuzzo et al., 2002) but it is still an equipment with difficulty accessible. The availability of a three-dimensional (3D) measuring technique coupled to specific image processing method opens new possibilities. SRμCT may provide 3D images with spatial resolution as high as one micrometer. The acquisition of 3D bone samples images at high spatial resolution using SRμCT has proved to be very accurate for quantifying human bone micro-architecture. Moreover SRμCT is a non destructive, fast, and very precise procedure to determine the DMB in 3D, simultaneously to the micro-architecture. The calibration procedure used homogeneous phantoms of water solutions at different concentrations of K_2HPO_4 (Nuzzo et al., 2002). This method was compared with the quantitative microradiography technique on the same bone samples, and showed that the values of the DMB are both in the range 0.5-1.6 g/cm^3 of bone, both in cortical and cancellous bone, with a mean difference around 4.7%, slightly higher in trabecular region (Nuzzo et al., 2002).

3.3 Bone microhardness at the tissue level

Another important characteristic of bone mineral is its hardness (Currey 2003; Nalla et al., 2003). Thanks to indentation techniques, it has been shown that microhardness of bone osteon was strongly related to its mineral content (Amprino 1958; Bala et al., 2010; Boivin et al., 2008; Carlstrom 1954; Weaver 1966). From a mechanical point of view, microhardness parameter is related to both elastic and plastic deformations, and an indentation technique has been developed to measure directly both elastic modulus (*E*) and contact hardness (*Hc*) on small area of bone tissue (Oliver & Pharr 1992). However, this technique has been developed for isotropic materials. While it is known that the bone tissue is complex with an anisotropic structure, the measurements of *E* is usually performed with a defined Poisson's ratio (ν=0.3). It has been shown that contact hardness was linearly interdependent with elastic modulus (Oyen 2006). Therefore, contact hardness can give an evaluation of bone stiffness which is directly related to its brittleness. Contact hardness can be evaluated at the microstructural (BSU) or nanostructural (lamellar) levels. At the microstructural level in bone, the pyramidal square-based Vickers indenter is often used (Fig. 6) (Boivin et al., 2008), and nanoindentation is rather used at the lamellar level using Berckovich indenter (Ammann & Rizzoli 2003). Very recently, it has been shown, by instrumented nanoindentation, that contact hardness was correlated both to DMB and collagen maturity (Bala et al., 2011b). Mineralization is a major determinant of microhardness, with about two-thirds of the variance, and one-third being explained by the organic matrix (Boivin et al., 2008). In human control bone, the microhardness does not vary with age and sex, in cortical and cancellous bone, as for the degree of mineralization. In 19 human control bones, the hardness in cortical bone is about 49.30 ± 2.16 kg/mm^2 and in cancellous bone about 48.92 ± 1.57 kg/mm^2.

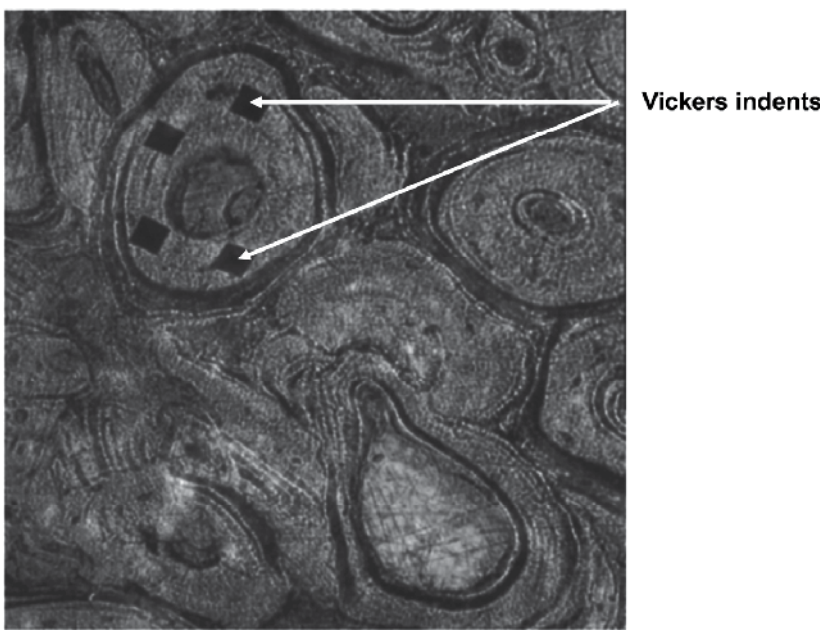

Fig. 6. Iliac bone from ewe showing a Bone Structural Units (BSU) with 4 Vickers indents (INSERM UMR 1033).

4. Characteristics of bone mineral crystals

Human bone mineral is a non-stoichiometric and poorly crystallized apatite. Bone apatite structure is hexagonal with space group $P6_{3/m}$, with lattice parameters a=9.42Å and c=6.88Å. It is a calcium (Ca)-deficient apatite analog, contains major elements like calcium, [Ca^{2+} (40 wt %)], phosphate [PO_4^{3-}(18 wt %)], carbonates [CO_3^{2-} (6-7 wt %)], minor elements such as magnesium (Mg^{2+}) or sodium (Na^{2+}), and trace elements (LeGeros & LeGeros 1983; LeGeros et al., 1968). Bone mineral also contains ions normally absent from body fluids (lead, fluoride, aluminium etc). Indeed, the apatite lattice is very tolerant to substitutions and vacancies. Compared to dental enamel, the bone mineral contains much more vacancies. In fact, apatite is able to incorporate itself, in its atomic structure, the half of the elements in the periodic chart (Wopenka & Pasteris 2005). Apatite lattice contains about 40 ions, and the unit cell is the smallest basic unit which is a sample of the entire lattice array (Glimcher 1998). In the apatite unit cell, four different types of crystallographic positions (or "sites") have been identified (Fig.7): (1) tetrahedral sites for six P^{5+}-ions, each in 4-fold coordination with oxygen, (2) Ca[1] sites for four of the Ca^{2+} ions, (3) Ca[II] sites for the six other Ca^{2+} ions, and (4) the channel site, occupied by two monovalent anions (OH^-, F^- and/or Cl^-) (Posner 1969; Wopenka & Pasteris 2005). The small ions (Cd^{2+}, Zn^{2+}, Mg^{2+}) are preferentially incorporated into Ca[I], whereas bigger ions (Sr^{2+}, Ba^{2+}, Pb^{2+}) are incorporated into Ca[II] (Fig. 7). The reason why apatite is the mineral component of vertebral skeleton is not known, but it was shown that apatite is the only calcium-phosphate mineral phase that is stable at both a neutral and basic pH (Glimcher 2006; Omelon et al., 2009). Another explanation is coming from the presence of denses granules containing polyphosphates near the mineralizing cartilage and resorbing bone (Omelon et al., 2009). Indeed, when the mineral apatite is dissolved after acidification and resorption by osteoclasts, there is no reprecipitation within the resorption pits, even the return to a neutral pH. The hypothesis of the authors was that polyphosphates formation provides a mechanism for accumulating phosphate, controlling the apatite at locations sites previously mentioned. Enzymatic action can thus control apatite supersaturation at neutral pH, directly by controlling orthophosphate ion activity (Omelon et al., 2009).

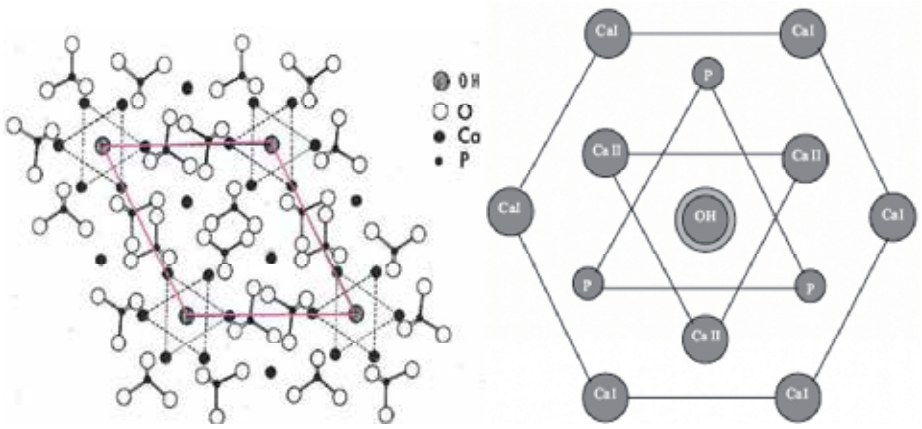

Fig. 7. (Left) Hexagonal system of apatite lattice, showing the disposition of atoms. (Right) The Ca ions occupy two crystallographic non-equivalent sites (Ca I and Ca II). From (Reprinted from Li et al., 2007, with permission from Elsevier). Small ions (Cd^{2+}, Zn^{2+} and Mg^{2+}) are preferentially incorporated into Ca[I], whereas bigger ions (Sr^{2+}, Ba^{2+} and Pb^{2+}) are incorporated into Ca[II].

4.1 Bone crystal size and shape

The crystal structure and morphology of bone minerals have often been controversial, mainly due to the different techniques used to characterize bone mineral. Today, with the use of more accurate method (atomic force microscopy, high resolution transmission electron microscopy), it is clear that the bone mineral crystals are very small and platelet-shaped (length ≈ 200-600 Å, width ≈ 100-200 Å, thickness ≈ 20-50 Å, Figs. 8 and 9). Compared to bone crystals, enamel crystals are needle-shape and much bigger. This small bone crystal size has several advantages. First, it permits an extended surface area (100-200 m^2/g). Two factors are involved in the surface activity: the surface area expressed in m^2/g and the physical and chemical properties of the surface. These properties determine the type of reactions, while the surface area determines the number of reactions. The combination of both factors makes the bone mineral substance metabolically very active; consequently, crystals have a very large interface with extracellular fluids. For example, the crystals contain in a small lumbar vertebra (L1 or L2) having a dry wet of 30 g, have a specific surface comparable to that of the playing field of soccer. Bone mineral is metabolically active, various and numerous interactions between ions from the extracellular fluid and ions constituting apatite crystals, are thus possible. Second, another interest of the small crystal sizes is mechanic. Indeed, the highly ordered location and orientation of very small crystals within the collagen fibrils permits an acceptable range of flexibility without fracture or disruption of the bone substance (Glimcher 1998; Landis 1995).

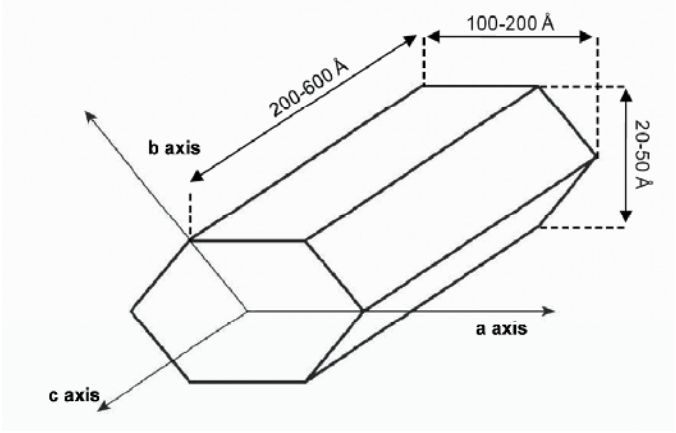

Fig. 8. Schematic representation and crystal size of bone apatite with the 3 axis (INSERM UMR 1033)

4.2 Relationships between water, organic matrix and mineral

In bone, the process of crystal nucleation in bone matrix is heterogenous, and is formed within the "hole" band of type I collagen (670 Å) (Glimcher et al., 1957). During the process of mineralization, the apatite crystals replaced some of the molecules of water so their content is inversely proportional to that of water (Elliott & Robinson 1957; Robinson 1975). Once deposited, the mineral phase induces compaction of the collagen fibril structure. Neutron and X-ray diffraction have shown that the Bragg-spacing of collagen strongly decreases with increasing mineral content (Lees 1987). Computer modeling and SAXS confirmed the process of closer packing of the collagen molecules when clusters of mineral

crystals replaced the water within the fibrils (Fratzl et al., 1993). The expansion of mineral crystals compressed the collagen molecule packing, thus decreasing the molecular spacing. This indicated the close relationship between water and the mineral deposition process. Modification of the collagen packing probably influences secondary structure of organic matrix. More recently, in intact bovine bone, the effects of dehydration have been studied using solid-state NMR spectroscopy (Zhu et al., 2009). Interestingly, well-resolved peaks broadened with dehydration, suggesting a conformational disorder and structural changes of bone matrix. This is in agreement with other studies showing a collagen conformational change with dehydration (Naito et al, 1994 ; Saito et al, 1984, 1992).

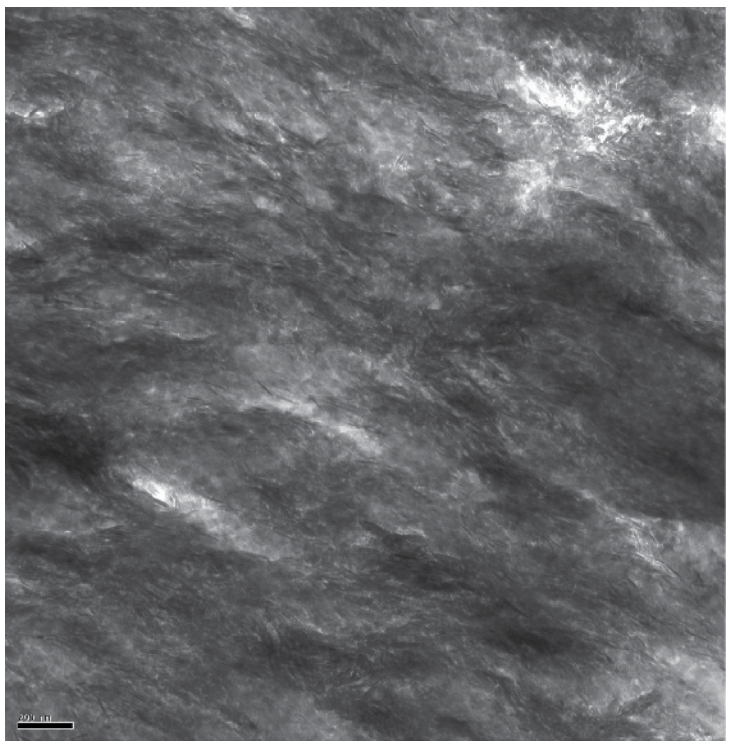

Fig. 9. Electron micrograph of human cancellous bone (woman, 80 year-old) illustrating the bone crystals within the type I collagen (INSERM UMR 1033).

It was suggested that water could play a role in the mechanical behavior of cortical bone (Nyman et al., 2006) and the removal of water alter crystallographic structure of synthetic apatites (LeGeros et al., 1978). As the water content decreases in bone with age (Jonsson et al., 1985; Mueller et al., 1966), it was suggested that water could be involved in bone fragility. Three main types of water exist in bone: the freely mobile water located into vascular lacunar canalicular spaces, the water bound to the collagen network, and the water bound to the mineral. More precisely, there is two type of water bound to the mineral: (a) the water bound to the surface of bone crystals, and (b) the water located within the apatite lattice. Water bound to the collagen fibrils provides post-yield toughness to bone and when water was removed, the strength and stiffness was increased whereas the toughness was decreased (Nyman et al., 2006). The role of the loss of water bound to the mineral on bone

strength was not clear in this study, while it was suggested that the loss of water located in apatite lattice could change the size of the bone mineral crystals (Nyman et al., 2006), since it was already observed in dehydrated enamel or precipitated apatites (LeGeros et al., 1978). Consequently, the decrease in bone strength and toughness related to age could be due to a change in water distribution.

4.3 Chemical composition of bone mineral

The composition of bone mineral was for a long time assimilated to hydroxyapatite, but it is not. Several studies showed the lack of OH^-, by inelastic neutron scattering, Raman of infrared spectroscopies (Loong et al., 2000; Pasteris et al., 2004; Rey et al., 1995b). Solid state-NMR shows that the percentage of OH^- does not exceed 20% of the amount expected in stoichiometric apatite in human cortical bone (Cho et al., 2003). In fact, the small crystal size could be one of the reasons for the absence of OH^- ions in the bone apatite. Indeed, Wopenka and Pasteris have suggested that the small crystal size and the great the atomic disorder within the unit cells of the crystal was not energetically favourable for apatite for incorporate OH^- into its channel (Pasteris et al., 2004). Another reason for the lack of OH-ions in bone apatite could be due to the type–B substitution of PO_4 by CO_3, creating a vacancy in the channel site to maintain electrostatic equilibrium.

Nature of apatite	Chemical formula
Hydroxyapatite	$Ca_{10}\ (PO_4)_6\ (OH)_2$
Bone mineral	$Ca_{8.3}\square_{1.7}\ (PO_4)_{4.3}\ (HPO_4)\ (CO_3)_{1.7}\ (OH)_{0.3}\square_{1.7}$
Dental enamel	$Ca_{9.4}\square_{0.6}\ (PO_4)_{5.4}\ (HPO_4)\ (CO_3)_{0.6}\ (OH)_{1.4}\square_{0.6}$

Table 1. Chemical formula of different apatites (□: vacancy) (adapted from Cazalbou et al., 2004a)

When an ion with the same electric charge is substituted within the bone apatite, no effect is produced on the structure lattice. If ions have a different electric charge (CO_3^{2-} substituted for PO_4^{3-}), a vacancy is created to maintained electrostatic equilibrium. Some ions can be replaced by other ions of almost identical radius. These ions induce only minor changes in shape at crystal level and do not affect the structure of the crystal. Such substitutions occur during formation of the crystal or through ionic exchanges with the existing crystals. In vitro, some interactions between mineral substance and the solution lead to the diffusion of ions within the hydrated shell, the exchanges at the crystal surface and the exchanges inside the crystal. Similar mechanisms are likely to occur in vivo during remodeling of bone tissue (Blumenthal 1990; LeGeros 1981; Posner 1985). Some cations of similar size and charge as the Ca^{2+} (Sr^{2+}, Na^+), as well as others that cannot substitute for the Ca^{2+} in the apatite structure (Ba^{2+}, Ra^{2+}, Mg^{2+}, K^+), are easily exchangeable from the solution to the surface Ca^{2+} ions. Such substitutions lead to modifications in the *a* and *c* parameters of the apatite unit cell. Substitutions with Mg^{2+} are only partial. In biomimetic apatite nanocrystal, the incorporation of Mg has been recently studied to analyze the effect of substitution with Ca^{2+} on particule morphology (Bertinetti et al., 2009). Incorporation of Mg^{2+} does not affect apatitic nature, nonetheless, a lower degree of crystallinity was observed by XRD (Bertinetti et al., 2009). Moreover, it was shown that apatites enriched with Mg^{2+} retain more water at their surface than Mg^{2+}-free apatites (Bertinetti et al., 2009). Calcium can be easily substituted by

significantly larger ions, but less frequently by smaller ions (Blumenthal 1990). An ion can only be substituted to another if its ionic radius is less than 10% higher than the radius of the ion replaced. The exchange of anions (PO_4^{3-}, F^-) around the crystal surface is also well known. The substitution of F^- with OH^- ions can not be reversed because it leads to the formation of a more stable compound: F^- ions are more similar to Ca^{2+} ions than OH^- ions and the electrostatic links between Ca^{2+} and F^- are stronger than the ones between Ca^{2+} and OH^-. The more insoluble apatite is the fluoroapatite [chemical formula: $Ca_{10}(PO_4)_6F_2$]. To be resorbed by osteoclasts, the bone mineral have to be more soluble than hydroxyapatite, and the vacancies present in bone mineral enable this dissolution. Carbonates can be found in apatite crystals as CO_3^{2-} ions, which can substituted for either PO_4^{3-} or OH^- ions. When the volume of CO_3^{2-} ions increases, the *a* parameter of the crystal unit cell decreases, while the *c* parameter increases.
Some foreign ions can increase or decrease the bone crystal size. In the case of Mg^{2+}, *in vitro* studies have shown that Mg^{2+} bound to the hydroxyapatite crystals retarded nucleation and growth of the crystal. In vivo studies show a decrease in crystal size in Mg-deficient rats, thus Mg interferes with the mineralization process (Bigi et al., 1992; Blumenthal et al., 1977; Boskey et al., 1992).
Others ions, as Fe^{3+} ions, have a direct effect on hydroxyapatite, inhibiting the growth and changing the quality of crystals (decrease in crystallinity and increase in carbonate substitution) (Guggenbuhl et al., 2008). Aluminium also affects bone mineralization, and osteomalacia renal osteodystrophy has been associated, in patients on long-term hemodialysis, with Al^{3+} accumulation in bone (Blumenthal & Posner 1984). A recent study on rats showed that a long-term Al^{3+} exposure reduces the levels of mineral and trace elements in bone (Zn, Fe, Cu, Mn, Se, B, and Sr) (Li et al., 2010b). This is accompagnied by a decrease in BMD especially in cancellous bone. An high amount of ions which are normally present in small proportion in bone mineral can cause alteration of bone substance. As previously mentioned, fluoride ions at high doses cause osteomalacia and defects of mineralization (Balena et al., 1998). On the other hand, small doses of some ions can have positive effect on bone strength. For example, Sr^{2+} (Strontium ranelate is an osteoporosis treatment) reduces both the vertebral and non vertebral fractures (Meunier et al., 2004; Reginster et al., 2005). Besides the effect of Sr^{2+} on bone cells (stimulating bone formation and decreasing bone resorption) (Grynpas & Marie 1990; Marie et al., 1993), the presence of Sr^{2+} is shown in the bone mineral formed during treatment, in osteoporotic women treated with strontium ranelate for 3-5 years (Boivin et al., 2010; Doublier et al, 2011a, b). The concentration of Sr^{2+} is very low, and do not exceed in human a maximum of 0.5 ions Sr^{2+} for 10 Ca^{2+} (Li et al., 2010a). Moreover, the thickness and length of the plate-shaped bone mineral crystals were not affected by the strontium ranelate treatment (Li et al., 2010a). Presence of Sr^{2+} causes no osteomalacia, no modification in the mineralization process or crystal size. However, the Sr^{2+} increases the bone strength, thus the presence of Sr^{2+} in bone mineral has certainly positive effects, but this mechanism is to date unknown.

4.4 Bone apatite: A particular structure, with a hydrated layer around an apatitic core

The surface of bone crystals, formed in the water of extracellular fluid, exhibits a "hydrated layer" (Fig. 10). Ions in this layer are very labile and reactive, and constitute the non-apatitic domain, surrounding the relatively inert and more stable apatite domain of the bone crystal (Cazalbou et al., 2004; Termine et al., 1973). Newly deposited bone mineral contains many labile non-apatitic domains [HPO_4, PO_4, and CO_3], located in the

well-developed hydrated layer involved in the high surface reactivity of mineral (Cazalbou et al., 2004). Labile PO_4 and CO_3 groups are easily and reversibly exchangeable with other ions in the hydrated layer. During maturation, the decrease in labile non-apatitic environments is associated with an increase in stable apatitic environments (Cazalbou et al., 2004). A particularity of the bone mineral is its non-stoichiometry, leading to the presence of numerous vacancies in the apatite crystal. Consequently, bone crystal is mainly maintained by electrostatic cohesion, thus bone crystals are easily soluble relative to stoichiometric apatite (Barry et al., 2002) . As bone becomes more mature, both the size and number of crystals increase.

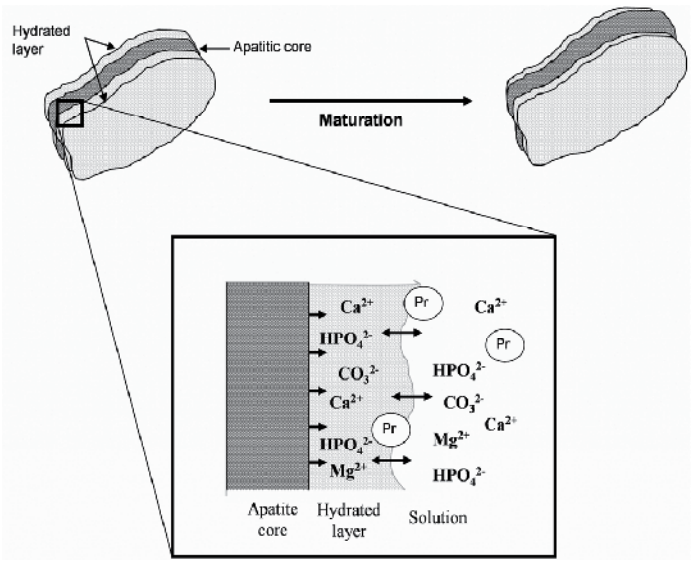

Fig. 10. Evolution of the hydrated layer and apatite core from bone crystal. During the maturation and growth of the crystal, the hydrated layer, involved in a high surface reactivity, progressively decreases and led to a stable apatitic domain. The structure of the hydrated layer constitutes a pool of loosely bound ions which can be incorporated in the growing apatite domains and can be exchanged by foreign ions in the solution and charged groups of proteins (Pr) (Adapted from Rey et al., 2009).

During mineral maturation, the hydrated layer decreases while the stable apatite domain grow, corresponding to the evolution of non-apatitic environments into apatitic environments detected by Fourier Transform InfraRed spectroscopy (FTIR). This hydrated layer, different from a hydration layer (Stern double layer), corresponds to the mode of formation of apatite crystals in physiologic conditions. The existence of these two domains (hydrated layer and apatite core) in biomimetic nanocrystals, has been recently confirmed by solid-state NMR (Jager et al., 2006). The hydrated surface layer contains loosely bound ions, which are easily exchangeable, and determine the surface properties of the nanocrystalline apatites (Cazalbou et al., 2004; Termine et al., 1973). In bone, those loosely bound ions can be also exchanged with charged groups of proteins present in collagen and non-collagenous proteins. The role of charged proteins on mineralization is well known (Boskey et al, 1989, 1998; Boskey 1989; Georges & Veis 2008; Landis et al., 1993; Malaval et al., 2008; Traub et al., 1992).

4.5 Measurement of mineral crystallinity

Crystallinity is defined as the degree of structural order in a crystal. The atoms in the crystal are arranged in a regular and periodic manner. The referent method to measure *absolute crystallinity* is the X-rays diffraction, and is based on the elastic scattering of the X-rays. This technique can be used to determine the crystal structure (Le Bail & Loüer 1978; Rietveld 1969) or chemical composition of a sample through the power diffraction bank data file (Powder diffraction file). The WAXS (wide angle X-rays scattering) is based on scattering angles 2θ larger than 5°, and SAXS (small X-ray scattering) gives informations on angles 2θ close to 0°. This method gives informations on the size, strain and orientation of crystals. A peak broadening can be due the small crystal size or microstrains. The crystal size can be determined by the *Scherrer* equation.

Vibrational spectroscopy techniques, such as Fourier Transform InfraRed Spectroscopy (FTIRS), Synchrotron InfraRed or Raman Spectroscopy, have been extensively used to study calcified tissues (Ager et al., 2005; Akkus et al., 2003; Boskey et al., 2005; Cazalbou et al., 2004; Farlay et al., 2010b; LeGeros 1981; Miller et al., 2001; Paschalis et al., 1996; Pleshko et al., 1991; Rey et al., 1990, 1991). Spectroscopic techniques allow assessment of physicochemical modifications of mineral induced by mechanical tests (Ager et al., 2005; Akkus et al., 2003; Carden et al., 2003; Morris & Mandair 2011; Tarnowski et al., 2004), age-related modifications (Ager et al., 2005; Akkus et al., 2003; Miller et al., 2007), and pathologic or treatment-related changes (Boskey et al., 2005; Carden & Morris 2000; Fratzl 2004; Huang et al., 2003; Siris et al., 2004). The application of Fourier Transform InfraRed Microspectroscopy and Imaging (FTIRM, FTIRI) for bone allows in situ analysis of embedded bone samples at the BSU level. These techniques are based on the vibrations of the atoms of a molecule and give complementary informations. The functional groups present in the sample absorb infrared light (or scatter light for Raman) at different wavelengths. In bone, infrared and Raman spectroscopies were used to obtain information on bone mineral and organic matrix, the two major components of bone. The main advantage of these techniques is that they can be used on embedded bone biopsies, on thin sections (2µm-thick MMA sections), keeping the integrity of the bone microarchitecture. By infrared spectroscopy, different vibrations can be analyzed in bone mineral: the phosphate mode vibrations with the $\nu_1\nu_3PO_4$ corresponding to an antisymmetric stretch, the ν_4PO_4 (bending stretch), the carbonate vibrations ν_2CO_3 and ν_3CO3. Organic matrix can be analyzed through the Amides I, II and III vibrations. Different parameters can be deduced from these vibrations, as the mineral maturity, crystallinity, carbonate substitution, mineral to matrix ratio, providing informations on bone mineral quality (Boskey et al., 1998, 2005; Miller et al., 2007; Paschalis et al., 1996, 1997a; 1997b; Paschalis & Mendelsohn 2000, Pleshko et al., 1991, Rey et al., 1989, 1995a). By Raman spectroscopy, crystallinity, carbonate type B substitution, and mineral to matrix ratio can be determined (Akkus et al., 2003; Morris & Mandair 2011).

Others techniques as vibrational methods have been extensively used to obtain informations on the structural and compound identification, especially on the relative crystallinity (also called crystallinity index).

4.6 Maturity and crystallinity of bone crystals

As mentioned previously, with maturation, stable apatitic domains grow, whereas hydrated layer decreases. Thus, when bone crystals mature, their crystallinity also increases. These two variables can be assessed separately in infrared spectroscopy (Fig.11). Mineral maturity and mineral crystallinity are two parameters temporally linked and often well correlated in

synthetic apatite. The ratio 1030/1020 cm^{-1} (apatitic phosphate over non apatitic phosphate, ν_3PO_4 vibration) was well correlated in synthetic apatites to the crystallinity measured by XRD, and it was established that the ratio 1030/1020 cm^{-1} was an index of mineral maturity/crystallinity. From that, mineral maturity and crystallinity were associated in a lot of studies (Boskey et al., 2005; Paschalis et al., 1996, 1997a, 1997b). We have defined a new ratio to assess mineral maturity, the 1030/1110 cm^{-1}, (apatitic phosphate over non apatitic phosphate, ν_3PO_4 vibration) equivalent to the 1030/1020 cm^{-1} but more sensitive (Farlay et al., 2010b). We agree with the fact that the 1020/1030 cm^{-1} ratio, which is an index of mineral maturity, evolves simultaneously with crystallinity in synthetic samples or normal bone. However, by definition, those two parameters are different, as mineral maturity corresponds to a stage of maturation, and crystallinity corresponds to the organization of the apatite lattice. We have defined a new mineral crystallinity index, measured by FTIRM on the peak 604 cm^{-1} (bending vibration of phosphates) (Farlay et al., 2010b). This vibration is often inaccessible for microscopic infrared imaging, due to the cut-off of the detector. A wide band detector allows the access to this vibration and we have shown that the value of the full width at half maximum of the 604 cm^{-1} peak, was inversely correlated to the crystallinity (Farlay et al., 2010b). This crystallinity index is well correlated, on the same samples, with another crystallinity index measured on the same vibration, the Shemesh ratio (Shemesh 1990) which is itself derived from the splitting factor of Termine & Posner, 1966. In human bone, it has been shown that those two parameters could evolve separately, and thus can independently affect the mineral characteristics. Indeed, mineral maturity can be affected by modification of bone remodeling (and by formative or antiresorptive treatments), whereas crystallinity can be influenced by ionic substitutions. This was verified in bone samples from patients with skeletal fluorosis (Farlay et al., 2010b). Skeletal fluorosis is a pathology caused by an excessive consumption of fluoride, and characterized by ionic substitution of hydroxyl ions by fluoride ions in bone mineral. In these samples, mineral maturity was decreased, due to a stimulation of osteoblasts on bone formation by fluoride, but crystallinity was increased due to the substitution of OH^- by F^-.

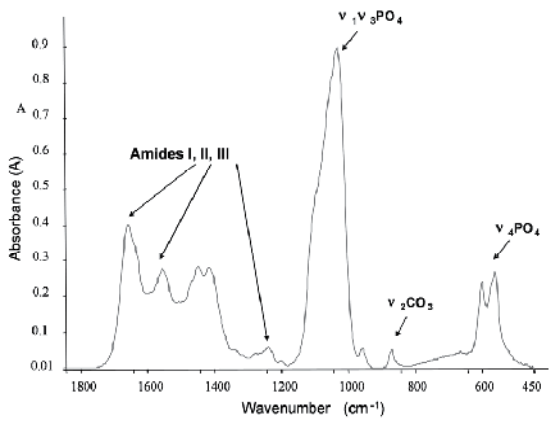

Fig. 11. Infrared spectra of human cortical bone (iliac bone) showing the mineral ($\nu_1\nu_3PO_4$, ν_4PO_4,ν_2CO_3) and the organic (amides) vibrations (INSERM UMR 1033).

In the study previously mentioned for determining the chronology of secondary mineralization on ewes (Bala et al., 2010), it has been shown that kinetic of mineral maturity

was very close to the kinetic of the degree of mineralization, whereas the crystallinity index was biphasic, showing a rapid increase for the six first months, then stabilizing until 18 months, and showing another increase toward highest values after 24 months (Fig.12). In another study performed in women long term-treated by bisphosphonates, an increase in mineral maturity was observed, associated with a decrease in crystallinity, reinforcing the statement that mineral maturity and crystallinity have to be cautiously separately analyzed (Bala et al., 2011).

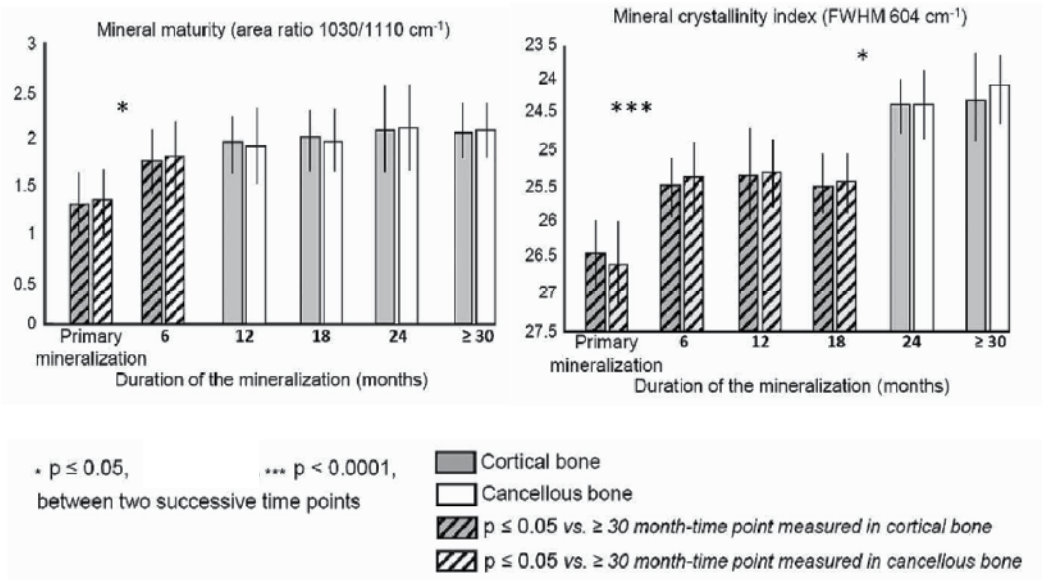

Fig. 12. Histograms showing (left) the rapid increase of mineral maturity during the 6 first months of mineralization, followed by a slowdown and stabilization; (right) the biphasic evolution of mineral crystallinity index, with an increase the 6 first months, a stabilization, and then a resumption at 18 months (Reprinted from Bala et al., 2010, with permission from Elsevier).

Finally, in a study on 53 human vertebrae, it was shown that with age of donor, crystallinity was increased and mineral maturity unchanged (Farlay et al., 2010a). This increase in crystallinity was previously shown in osteoporotic bone (Boskey, 2003), suggesting that with aging, an increase in crystal size/perfection occurs, independently of the bone remodeling (Farlay et al., 2010a).

4.7 Carbonates in bone mineral

In bone mineral, carbonates ions represent about 6-7% of the total mineral ions, thus a non negligible proportion. CO_3^{2-} can be incorporated into bone mineral by substitution in the apatite lattice either to PO_4^{3-} (major site, type-B carbonate) or to OH^- (minor site, type-A carbonate). A third site of CO_3^{2-} ions corresponds to labile carbonates which decrease with the maturation of the apatite crystal (Rey et al., 1989, 1991). The carbonates decrease the regularity of the atomic arrangement in hydroxyapatite (Blumenthal et al., 1975), thus altering the crystallinity. CO_3^{2-} determine physical properties of materials, or biological behavior of cells in scaffold or ceramics. The CO_3^{2-}A/B ratio is very constant among

different species, suggesting that CO_3^{2-} incorporation in bone is a highly regulated process. Carbonates are also important for mineral dissolution (LeGeros 1981). It is well-established in literature that, in synthetic apatites, CO_3^{2-} content increases with time of maturation (Cazalbou et al., 2004b; LeGeros & LeGeros 1983; LeGeros et al., 1968; Rey et al., 1989, 1995a). However, in bone, there is a controversy with some studies showing an increase of CO_3/PO_4 with mineral maturation (Petra et al., 2005) or a decrease (Farlay et al., 2006; Ou-Yang et al., 2001; Paschalis et al., 1996). In very young bone, labile carbonates and HPO_4 are high, and progressively decreased with time of maturation, this being associated with an increase in mineral crystallinity (Magne et al., 2001). The amount of carbonate ions can be measured by different methods (chemical dosage, thermogravimetric analysis, vibrational spectroscopies…). In infrared spectroscopy, carbonate content is generally determined using the ν_2CO_3 line (out-of-plane bending vibration) which is free of contribution of amide vibrations, and was generally calculated as CO_3/PO_4 area ratio. The role of carbonate in bone mineral is not entirely elucidated. The hypothesis of Wopenka and Pasteris is that carbonate could control bone crystal size, and that high concentration of carbonate could play an important role in constraining bone crystals to the nanometer scale (Wopenka & Pasteris 2005). Indeed, dentin, in which the amount of carbonate concentration is the same than in bone, has also a crystal size similar to bone. However, enamel crystals, which are very much larger size, have only about half the carbonate concentration as bone does.

5. Modifications of bone mineral characteristics with age or in post-menopausal osteoporosis

Bone loss occurs in all individuals after middle life. This bone loss can be moderated (osteopenia), or can become more important (osteoporosis). Osteoporosis is a metabolic bone disease, leading to a decrease in the amount of mineralized bone and an increase in the risk of fracture. In post menopausal osteoporosis, the oestrogen deficiency lead to an acceleration of bone remodeling, the balance between resorption and formation is disturbed in favour of resorption. This lead to a decrease in the more mineralized bone, which is only partially replaced by young less mineralized bone, reducing material stiffness.

5.1 Modifications of bone mineral at tissue level

Several studies performed in bone from osteoporotic women have shown not only that bone mass is decreased but that the bone mineral content was decreased, due to the fact the newly formed BSUs have not the time to achieve their mineralization before to be resorbed. Thus the mean age of matrix is decreased, leading to a decrease to the mean degree of mineralization.

5.1.1 Degree of mineralization (DMB) in control humans (Boivin et al., 2008)

Bone samples from persons of both sexes (sudden died and without known pathology) were studied. This control group was composed of iliac bone samples taken at necropsy form 30 women (aged 48.4 ± 3.7 years; range 20-93 years) and 13 men (aged 66.0 ± 4.4 years; range 43-86 years). The mean DMB expressed in g mineral/cm^3 (mean ± SEM) was 1.082 ± 0.017 in cortical bone and 1.099 ± 0.018 in cancellous bone (Figure 13).

In iliac crests, no significant influence of sex or age on the mean DMB was observed (Boivin et al., 2008). In cancellous bone, another study by qBEI showed the absence of influence of

sex, age, ethnicity or skeletal sites in BMDD measurements (≈22% in w% Ca) (Roschger et al., 2003). A recent study on vertebral bone of 53 donors (age range: 54-95 year-old, 21 men, 27 women) showed no influence of sex and age on the DMB (Follet et al., 2010).

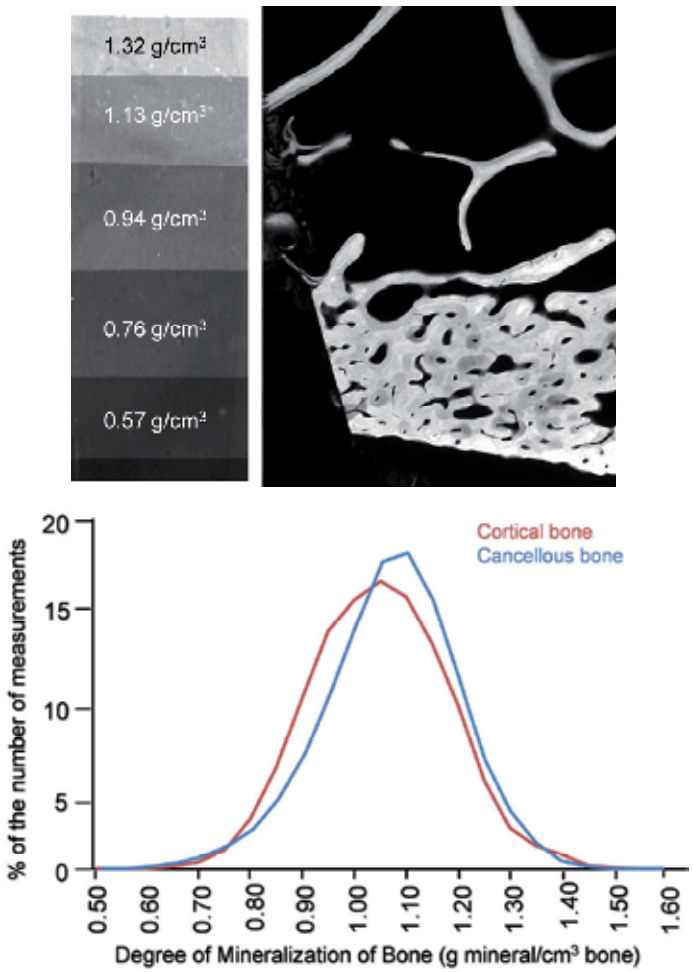

Fig. 13. Quantitative microradiography: (top) aluminium step wedge and microradiograph of bone tissue. (bottom) Distribution of the degrees of mineralization in human iliac bone (INSERM UMR 1033).

5.1.2 Bone mineralization at tissue level in bone of post-menopausal women treated with anti-osteoporotic treatments

In adult bone, the DMB, i.e., the bone mineral density at the tissue level depends on the rate of remodeling. Thus, agents (parathyroid hormone) or events (menopause, ovariectomy) which provoke an augmentation in the « birthrate » or activation frequency of Basic Multicellular Units (BMUs), induce a decrease of the « lifespan » of BSUs, in other words on the time available for the secondary mineralization. This leads to the fact that new BSUs are resorbed before they have fully completed their secondary mineralization, as proven by the presence of a large amount of uncompletely mineralized BSUs and a low mean DMB (Arlot

et al., 2005; Boivin & Meunier 2003; Misof et al., 2003). In a study on men and women with idiopathic osteoporosis, a decrease in DMB was observed compared to controls (-7%) (Boivin et al., 2008). However, a decrease in heterogeneity index of mineralization was observed in this study, indicating a homogeneization of the mineralization. This decrease is also accompanied, in men only, by a decrease in microhardness (-10%) (Boivin et al., 2008). However, it is important to mention that, both in control or osteoporotic bone, no variation in DMB or microhardness was correlated with age. Conversely, antiresorptive agents (bisphosphonates, calcitonin, estrogen, SERMs) which cause a marked reduction in the « birthrate » of BMUs, prolong the « lifespan » of the BSUs, allowing a more complete secondary mineralization and an increase of DMB (Bala et al., 2011a, 2011b; Boivin et al., 2000, 2003; Borah et al., 2005, 2006). A dissociating agent (Strontium Ranelate), acting both on resorption and formation, is now used in the treatment of post-menopausal osteoporosis (Meunier et al., 2004; Reginster et al., 2005). After short- and long-term treatment, the DMB was not significantly different from the physiological range (Boivin et al., 2010; Doublier et al., 2011a). Recently, also with a microradiographic method, it has been shown, in hip-fracture patients, an increase in DMB heterogeneity in both interstitial and osteons compared to controls (Bousson et al., 2011). Thus, some differences exist, considering osteoporotic or hip-fractures cases, in term of heterogeneity of mineralization.
The mineralization index measured by FTIRM, calculated as the ratio of $\nu_1\nu_3PO_4$/amide I vibration (Paschalis et al., 1996) is a relative index which has been correlated to ash content (Faibish et al., 2005) and DMB (Farlay et al., unpublished data). The mineralization index (or mineral to matrix ratio) has been shown to be higher in bone of post menopausal woman treated with alendronate (Boskey et al., 2009).

5.2 Modifications of bone crystal characteristics

Concerning crystal characteristics, it has been shown, by XRD technique that, with age, there was an increase in crystal size. In 117 iliac bone samples (range of age: 0-90 years), there was an increase, between 0-30 years, in crystallinity in c-axis, corresponding to the crystal length, and a decrease in the a axis (Hanschin & Stern 1995). After 30 years, modifications were less evident, only a slight increase in crystallinity within the basal plane was observed.
Modifications of bone crystal size impaired mechanical characteristic of bone. Indeed, it was observed that the bones of older animals or that osteoporotic bone, two cases in which bone fractures easily, contained more large crystals than normal (Boskey 2003). Conversely, young animals, which have mechanically strong bones, have a mixture of small and large crystals. In a recent study performed by Raman microspectroscopy in human femurs (age range: 52-85 years old), an overall reduction in heterogeneity of mineralization, crystallinity, and type-B carbonation was observed (Yerramshetty et al., 2006). In this study, bone became more mineralized and more highly type-B carbonated with age, whereas crystallinity was unchanged. In a study performed by FTIRM on 53 human vertebrae (range of age: 54-93 years), a significant increase in crystallinity was observed (Farlay et al., 2010a). This means that, in vertebrae, either crystal size or crystal perfection is increased. In those vertebrae, the bone volume was also significantly decreased. The reason of the increase in crystallinity is unclear, but it is possible that it corresponds to a mechanism of adaptation of bone, in order to compensate the bone loss due to age. In the same study, a decrease in CO_3/PO_4 ratio was observed suggesting an alteration of the physico-chemical composition of bone mineral with age. Thus, comparing the results obtained in human femora and vertebrae in term of

physico-chemical composition, some differences appears. However, the techniques were different (FTIR and Raman microspectroscopies), and the bones were not extracted from the same donors.

6. Perspectives and conclusion

Different factors increase the risk of fracture, independent of bone mineral density. Among these factors, bone mineral features are important in determining some mechanical behavior, especially elastic properties. Besides the decrease in bone mineral volume occurring with age, the alteration of mineral quality plays also a major role in bone fragility. The study of bone mineral quality is a instrumental challenge, due to [1] the dimension and the nature itself of crystal apatite (nanocrystalline), [2] the fact that bone is a composite material (organic matrix intimately linked to bone mineral), and [3] because bone is a complex tissue with different levels of organization (organ, BSU, crystal).

Thus, the using of different complementary techniques (spectroscopic, diffraction, electron microscopy techniques) has been very useful in the comprehension of bone mineral structure the last 50 years. Despite very important findings, there are still lacks in the understanding of the role of certain ions (minor or trace elements) in the biological or biomechanical properties (such as carbonates, strontium ions etc.).

Bone is a fascinating tissue governed by the remodeling activity and the plasma composition. It underlies several characteristics which are interdependent. For example, carbonate ions substitutions into apatite promote the platelet-shape in bone crystals rather than needle-shape found in enamel. But the presence of carbonates, moreover, as mentioned previously, inhibits the incorporation of OH^- in bone apatite. Thus, the study of correlations between different variables is still indispensable to understand the relationship between physicochemical and mechanical properties.

From the previous studies performed on bone mineral, it seems that a small crystal size associated with a heterogeneity of crystal size (nanometer size-scale) is important to an optimal mechanical strength. Indeed, the presence of too large crystal is not good for bone strength. Fluoride, as mentioned previously, induces the formation of large crystals, and the formation of a “brittle” bone, despite a high BMD. Moreover, concerning mineralization and its heterogeneity at the tissue level, values comprised between 0.8 and 1.30 g/cm^3 of bone could be a great deal to maintain stiffness of the bone. The preservation of mineralization heterogeneity is necessary for bone mechanical strength, allowing, for example, the stopping of microcracks propagation. No modification in degree of mineralization is observed with age or sex, but some differences exist in hip- fracture or osteoporotic cases. Thus a modification in the calcium absorption or an increase in the bone remodeling can lead to a decrease to the degree of mineralization. Differences also exist in studies in degree of mineralization due to the fact that either weight or non-weight-bearing bones are analyzed. We have an ongoing study on these different types of bone (weight or non-weight-bearing bones) in a same donor, in order to analyze the structural and mechanical bone mineral properties.

In conclusion, despite a lot of studies have permitted to understand the characteristics of bone mineral, further studies are needed to clarify the mechanisms of bone fragility at the different levels of investigation.

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2

Genetics and Osteoporosis

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1. Introduction

Osteoporosis is a multifactorial disease influenced by multiple factors and characterized by an imbalance in the regulation of bone remodeling that cause microarchitectural deterioration which compromises the bone strength and leads to bone fragility increasing the fracture risk. Since several years ago, the World Health Organization has considered osteoporosis as one of the most important public health issues worldwide, with a great repercussion in patients' life quality and in their familiar, social and work environments. Osteoporosis is an important problem in Latin America, currently its prevalence is similar to that in South Europe and slightly lower than in North Europe and among white population in the USA; World Health Organization estimates that in the forthcoming 50 years, osteoporosis prevalence will increase in Latin America until reach those of the currently observable in Europe and USA (World Health Organization [WHO], 1994; National Institute of Health [NIH], 2001 Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy; Cole ZA et al., 2008). During the last decades, the life expectancy has been increased notoriously and the number of subjects older than 60 years old has been increased. This situation in combination with the adverse environmental conditions and the life style, will cause a notorious increment in the incidence of chronic-degenerative diseases in the next decades, as will occur with osteoporosis. Certainly, primary osteoporosis use to be more frequent in posmenopausal women (Greespan et al., 1993); however occasionally it appears in premenopausal women which present several risk factors and even males may be affected by this disorder. It is important to mention that the actual life style favours the inadequate bone quality of children and young people (Asociación Mexicana de Metabolismo Óseo y Mineral, 2001). There are two forms of osteoporosis; primary OP, named posmenopausal or senile form and secondary OP, which is related to diverse endocrine, renal, rheumatic and genetic diseases, and with the prolonged administration of some drugs which induce bone loss (Riggs et al., 1986; Elliot-Gibson et al., 2004).

Discussing about osteoporosis it is necessary to mention the term "peak bone mass" (PBM), which refers to the maximum bone mass that an individual reaches in his life and it occurs between 20-30 years old approximately. PBM is the result of the interaction of multiple genetic and environmental factors; upon the PBM is reached, progressive loss of bone mass occurs naturally, depending on the magnitude and speed of subsequent bone loss (Burclar et al., 1989; Kanis et al., 1994; Guéguen et al., 1995.). The annual average bone loss in posmenopausal women is estimated in 1-2%, and 0.2.-0.5 % in males. It is considered that

about 30% of women at this phase shows an accelerated bone loss (approximately 5% per year) during the first 5 years after menopause, which represents higher risk to suffer osteoporotic fractures at this moment of their lives (Elliot –Gibson V et al., 2004).
Osteoporosis has been characterized for having a very discrete clinic behavior; it is practically "silent" remaining latent for years or could get worsen without causing significant symptoms. Nevertheless, one of the most frequent clinic manifestations is the back chronic pain, which may be attributed to the presence of vertebral micro-fractures, frequently it can be noted progressive height loss due to vertebral compression and/or slimming; this anomalies can be heterogeneous and cause loss of the spine natural conformation causing abnormal curvatures and scoliosis (Ismail et al., 1999). Fractures are the most frequent and dangerous complication of osteoporosis and may occur practically in all bones, even with a discrete trauma and spontaneously. As has been documented in LAVOS (*Latín American Vertebral Osteoporosis Study*) (Clark et al., 2009). and EVOS (European Vertebral Osteoporosis Study) (Raspe et al., 1998) studies, the spine is the most common site in which fracture occur. Booth studies showed that, the frequency of these fractures are related to gender and age, but also to races geographic distribution. Apparently they are more common in Scandinavian and North American population, whereas they are less frequent in South of Europe. Interestingly, the frequency is higher in urban areas than in rural ones, which outstands the importance of environmental factors in this disease besides of the genetic predisposition. After vertebral fractures, hip fractures occur, followed by forearm. It is estimated that about 25% of individuals showing this kind of fractures die due to complications, and other 25% (even the after the surgery), never recover the life quality they have before the fracture. On the other hand, patients who have suffered one or more fractures (in any place) predispose to have new fractures, independently of their bone mineral density (BMD). The risk for new fractures is higher in individuals who have suffered first fractures at early age and in those who have higher number of previous fractures.

2. Genetic susceptibility in osteoporosis

There are several elements that suggest that bone phenotype is under of an important genetic influence. The first observation is the familial aggregation detected in the clinical practice, in which can be observed the segregation of some phenotypic characteristics, like family history of bad bone quality of osteoporotic fractures (Guéguez et al 1995; Fox et al., 1998; Kannus et al., 1999). On the other hand, description in literature of several diseases of genetic origin with monogenetic inheritance, which phenotype includes the loss or gain of mineral bone density, supports the hypothesis that bone phenotype has an important genetic component. Some of the most studied diseases are the different forms of osteogenesis imperfecta, the diverse varieties of osteopetrosis, pyknodisostosis, sclerostenosis and osteoporosis syndrome accompanied by pseudoglioma (Barros et al., 2007), among others. Besides, there are reports of severe osteoporosis cases in which mutations have been detected in genes which have been previously associated with the genetic control of mineral bone density, as the genes for estrogens receptors 1 and 2 (ESR1, ESR2), androgens receptor (AR) and vitamin D receptor (VDR). Changes in the normal sequence of those genes could cause osteoporosis. However, the primary osteoporosis represents the most common form in all populations (Duncan et al., 2005, 2008, 2010). Primary osteoporosis has a multi-factorial and polygenic origin and the evidences that it shows clearly genetic susceptibility are family history of bad bone quality and fractures,

familial or demographic similarity during the natural history development of the disease or even differences in the pharmacological management response. In accordance to National Osteoporosis Foundation (IOF) 2008 statements, fractures family history represents an important risk factor independently of the bone mineral density and the presence of osteoporosis in first degree relatives has been related to the decrease in peak bone mass.

The analysis of genetic susceptibility to osteoporosis has been complicated because it is caused by the effect of multiple genes that exert their effect on the bone phenotype, taking in account that a great number of environmental factors acting on BMD are involved; however, despite all these difficulties, a large amount and variety of worldwide investigations suggest that BMD heritability ranges between 40-70% in spine, between 70-85% in hip and between 50-60% in wrist (Andrew et al., 2005; Michaelsson et al 2005; Deng et al., 2002). Densitometric studies in monozygotic twins (MC) and dicygotic twins (DC) have revealed that spine and femoral neck BMD consistency is higher (6-8:1) in MC twins than in DC twins. Family studies have estimated that fractures heritability ranges between 20-60%, depending on the anatomic region where those occur (Michaelsson et al 2005; MacGregor et al., 2000; Deng et al., 2002). In these cases, classic segregation studies have facilitated identifying new genes related to the BMD genetic control.

In the other hand, association studies have also been very helpful to associate particular phenotypic characteristics, such as bone mineral density or the occurrence of fractures, with very specific genetic variants (gene polymorphisms, specially single nucleotide variants). Besides, there are other bones characteristics with evident heritable component, among them are: geometry and length of the femoral neck, bone ultrasonic properties (which represent the trabecular interconnectivity degree), growth and speed of bone remodeling, bone dimensions and other conditions that have an impact on bone quality (Slemenda et al., 1996; Arden et al., 1996); for example body mass index and age at which menopause occurs. It is convenient to mention that family history of hip fractures has consistently been shown to be a risk factor for osteoporosis(Andrew et al., 2005).

The functioning of osteoarticular system is extremely dynamic and complex, it is constantly under remodeling and it have multiple and varied mechanisms to maintain homeostasis; therefore, its genetic regulation mechanisms are also complex to understand and integrate. Genes that have been linked with BMD genetic control are distributed along all the human genome and, they are in practically all chromosomes, each of them fulfills different functions and contributes in a different way to the genetic control of bone phenotype (Stewart et al., 2006; Xiong et al., 2006; Marini et al., 2010). There are some genes with important roles in bone homeostasis because their products are involved in elemental functions related to bone structure and metabolism (formation, growth, differentiation, resorption, maintenance, etc.) (Ralston et al., 2002; Williams et al., 2006).

Since long time ago, we know that bone metabolism has a great hormonal influence; therefore, genes that encode for its receptors are elemental in bone metabolism genetic regulation, among them we have genes ESR1 and ESR2 which encode for estrogens α and β receptors and are expressed in various bone cells types (osteoblasts, octeocytes and osteoclasts), both receptor types show a different expression pattern in the cortical and trabecular bones. Estrogens represent one of the most important regulators for bone metabolism, they regulate bone growth and maturation, and they also influence the differences between bone maturation and bone consolidation in men and women. These hormones have the capacity to block the osteoclastogenesis process, can interfere with the function of osteoclasts, induce them to

apoptosis, and may also modify the expression of genes involved in the bone remodeling process (Slemenda et al., 1996; Kameda et al., 1997; Cummings et al., 1998)). Moreover, hormones contribute to down the expression of the Tumoral Necrosis Factor (TNF), and thereby reducing osteoclasts response to the RANK and RANKL activity (the ligand binding to the activator receptor for the kappa B factor and its ligand) (Hughes et al., 1996).

It is already known that vitamin D, through the interaction with its receptor, plays an important role in calcium homeostasis for the regulation of growth and differentiation of bone cells; that is the reason why the gene that encodes for the vitamin D receptor (VDR) is quite important in bone metabolism. Another important gene is IL6, which codifies for interleukin 6, which is a proinflammatory cytokine that has been related to several biologic processes, as bone resorption, osteoporosis and other diseases as rheumatoid arthritis, diabetes mellitus, cardiovascular diseases, cancer, etc. LRP5 gene, which encodes for protein 5 related to the low density lipoprotein receptor that participates in the development and maintenance of several tissues and represent one of the regulators for the development and proliferation of the osteoblasts (Gong et al., 2001). Other genes relevant for bone metabolism are RANK, RANK-L and OPG which encode for key proteins for bone remodeling process (Capellen et al., 2002). Other genes with higher impact on bone phenotype is the COL1A1 gene, which encodes for one of the most abundant structural proteins in bone (collagen 1A1). A great number of investigations have analyzed the association among osteoporosis and allelic and genotypic variants of these genes (Ralston et al., 2002).

There are some characteristics, for example the body mass index, that could have an impact on bone phenotype. These traits are also under genetic influence so we found genes that are related with more than one phenotype. Since several years ago it is clear that there is an important relation between bone mineral density and body mass, we already know that overweight individuals should support higher weight opposite to individuals with a lower body weight, therefore, bone mineral density is higher in overweight subjects, while thinner subjects, including the ones with alimentary disorders as anorexia or malnutrition, could present low bone quality. Some of the genes with impact on these phenotypes are ESR1, ESR2, VDR, LRP5, IL6 and OPG between others (Deng et al-. 2002; Jie et al., 2009; Frenkel et al., 2010)K. During the last years, the leptin gene and its receptor (LEP and LEPR) have been revealed as an important hormonal factors for the regulation of appetite and energetic metabolism; besides, leptin has an osteogenic effect by stimulating osteoblasts formation and plays a direct osteogenic role on bone marrow stromal cells, which allows its differentiation and maturation to osteoblasts (Esteppman et al., 2000).

Other important genes in both phenotypes are the proinsulin gene (INS), its receptor (INSR), and probably too the gene family of growth factors similar to insulin, since apparently insulin exerts a mitogenic effect on osteoblasts, which could partially explain bone mass increment that is usually noticed in obese individuals.

Table 1 despicts some of the genes related to the bone phenotype and the function that has been attributed to their products. It is evident the genetic influence on different aspects of metabolism and homeostasis of bone tissue (structure, formation, resorption and remodeling) and the number of genes involved is large and their functions are diverse. In the case of bone structure highlights the COL1A1 and COL1A2 genes which code for the type I colagen protein, which represents over 90% of the organic matrix of bone. The osteocalcin and osteopontin are also important, the first one is a calcium binding protein which is secreted by osteoblasts and is encoded by the gene OC, while the phosphoprotein known as osteopontin, encoded by the gene OPN, is essential in the mineralization process.

Hormones and their receptors		
Gene	**Chromosomal location**	**Product**
ESRα	6q25	Estrogens receptor α
ESRβ	14q22	Estrogens receptor β
AR	Xq11	Androgens receptor
VDR	12q12	D vitamin receptor
PTH	11p15	Paratohormone
PTHR1	3p22	Paratohormone receptor 1
CT	11p15	Calcitonin
CTR	7p21	Calcitonin receptor
CYP1A1	15q21	Aromatase
CASR	3q13	Receptor sensitive to calcium
ADPN	3q27	Liponectin
GR	5q31	Glucocorticoids receptor
PRL	6p22	Prolactin
LEP	7q31	Leptine
LEPR	lp31	Leptine receptor
INS	11p15	Insulin
INSR	19p13	Insulin receptor
Matrix components		
COL1A1	17p21	Collagen 1A1
COL1A2	7q22	Collagen 1A2
OC	1q25	Osteocalcin
OPN	4q21	Osteopontin
With participation in osteoblastogenic processes		
ALOX12	17p13	Araquinodate 12 lipoxigenase
ALOX15	17p13	Araquinodate 15 lipoxigenase
BMP2	20p12	Morphogenetic protein of bone 2
BMP4	14q22	Morphogenetic protein of bone 4
BMP7	20q13	Morphogenetic protein of bone 7
IGF-1	12q22	Growth factor similar to insulin
LRP5	11q13	Receptor related to lipoprotein of low density 5
LRP6	12p13	Receptor related to lipoprotein of low density 6
SOST	17q12	Sclerotin
NOG	17q22	Protein antagonist of morphogenetic proteins

With participation in osteoclastogenesis processes		
Gene	**Chromosomal location**	**Product**
P53	17p13	Tumor suppressor P53 protein
CPK	1q21	Catepsine K
OC	1q25	Osteocalcin
OPN	4q21	Osteopontin
OPG	8q24	Osteoprogeterin
RANK	18q22	Receptor activator of NF-KAPPA-B
RANK-L	13q14	Ligand of the receptor activator of NF-KAPPA-B
CLC7	16p13	Chlorine channel 7
Cytokines and their receptors		
IL1α	2q14	Interleukin 1A
IL1β	2q14	Interleukin 1B
IL6	7p21	Interleukin 6
TNF	6p21	Tumoral necrosis factor
TNFR2	1p36	Tumoral necrosis factor receptor 2
Others functions		
MTHFR	1p36	5,10-Methylenetetrahydrofolate reductase
APOE1	19q13	Apolipoprotein E
MMP-1	11q22	Metalloproteinase
MMP-2	16q13	Metalloproteinase
MMP-9	20q11	Collagenase
PON-1	7q21	Esterase
SHH	7q36	Hedgehog protein (it participates in skeleton embryogenesis)

Table 1. Genes related to bone phenotype, their chromosomal location and their products

The osteoclastogenesis and the osteoblastogenesis are fundamental processes for the homeostasis of bone tissue as the speed and intensity of bone formation and bone resorption depending on several conditions. Both mechanisms show a significant genetic influence, so the amount of genes and therefore of proteins with participation in both processes is very significant. Among them are genes that encode for the family of bone morphogenetic proteins (BMP´s), the LRP5 and LRP6 genes that code for receptors for low density lipoproteins, which are involved in the osteoblastogenesis most likely to regulate the level of bone mineralization. The osteoclastogenesis is determined by the differential expression of genes of the RANK/RANK-L/OPG route. The P53 oncogene which product is very important for multiple biological processes and the cathepsin K gen (CPK) wich codes for a

collagenase with preferential expression in osteoblasts, indubitably play a crucial role in bone resorption.
Different hormones involved in the bone formation and remodeling, including the sex hormones (estrogen, progesterone, androgens), growth hormone, insulin, parathyroid hormone, calcitonin, cortisol and thyroid hormones. These hormones are implicated in different ways in bone metabolism according to the different stages, including intrauterine life, in such a way that the different hormones impact on linear growth of bones, bone maturation, bone homeostasis and the size that will be achieved in adulthood. That's why there are hormonal conditions such as hypothyroidism, hyperthyroidism, postmenopause, andropause and glucocorticoid prolonged intake which are capable to impact on the quality of the bone. Finally we can not ignore that various interleukins, growth factors and their receptors have been identified and the participation in the genetic control of bone mineral density of other proteins are still under study, as in the case of ILα, ILβ, IL6, TNF, TNFR2 among others.
On the other hand, it is important to mention that during the last years some investigations have pointed out that some of the genes related with bone phenotype have been related to other disorders as cardiovascular diseases; for example, genes such as osteoprotegerin (OPG), the receptor activator for nuclear factor kappa B ligand (RANKL) and bone morphogenetic protein 2 (BMP) have been associated with osteoporosis and with cardiovascular diseases, particularly atherosclerosis, which suggest that products of these genes take part in the calcification process (Collin-Osdoby et al., 2004; Marini et al., 2010).

3. Linkage analysis as strategy in the study of osteoporosis

Linkage studies are well validated for identification of responsible genes in monogenic diseases, since the inheritance of marker alleles is related to the inheritance of a bone trait within family members. Combining the use of statistical approaches in quantitative trait loci (QTL) and genome-wide association studies (GWAS), it is possible to establish a strategy to identify chromosomal regions which contain regulating genes of some important traits in complex polygenic diseases with genetically heterogeneous traits as osteoporosis, making possible to evaluate how many of the hundreds of proposed candidate genes are really associated. Most of linkage studies in osteoporosis selected the bone mineral density as the trait of interest; however regions that regulate other relevant phenotypes, such as bone mass and skeletal geometry, have been investigated.
Former studies identified important loci linked to bone mass and geometry. A genome search study in sib pairs recruited from families with a history of osteoporosis, obtained data suggestive of linkage of 1p36, 2p23-24 and 4q32-34 with spine and hip BMD (Devoto et al., 1998; Devoto et al., 2001). Studies with healthy female sib pairs demonstrated linkage of locus 11q12-13 with BMD variation (Koller et al., 1998) and evidence suggestive of linkage of 1q21-23, 5q33-35 and 6p1-12 to femoral neck or lumbar spine BMD was obtained in a genome-wide search study performed in Caucasian and African-American healthy female sib pairs (Koller et al., 2000). Other study identified loci in 5q and 4q that showed linkage to regulation of important aspects of femoral neck geometry (Koller et al., 2001). A QTL not previously described in 22q11 showed suggestive linkage in a study with families from Belgium and France (Kaufman et al., 2008). The presence of genes controlling BMD on 1p36 was suggested too in a multivariate linkage analysis in osteoporosis pedigrees (Zhang et al., 2009). One genome-wide scan for bone loss showed that change in femoral neck BMD in Mexican-American families is significantly linked to 1q23 (Shaffer et al., 2009). Interestingly

a study with pairs of brothers suggested that QTL on 7q34, 14q32 and 21q21 were male-specific (Peacock et al., 2009) and other report provides evidence of gender specific QTL on 10q21 and 18p11 (Ralston et al., 2005). Suggestive evidence of linkage of novel regions related with BMD and hip geometry on chromosomes 4, 5, 11, 16 and 20 was obtained in a sample of Caucasian Europeans (Karasik et al., 2010).

Two important large scale studies with a cohort of more than 19,000 european subjects, identified SNPs in previously proposed osteoporosis candidate genes and in regions not previously associated with femoral neck and lumbar spine BMD. SNPs from ESR1, LRP4, ITGA1, LRP5, SOST, SPP1, TNFRSF11A, TNFRSF11B AND TNFSN11 associated with either femoral neck or lumbar spine BMD in a cohort of more than 19,000 subjects. In the same study, SNPs from LRP5, SOST, SPP1 and TNFSF11A, were associated with fracture risk (Richards et al., 2009). The other study, confirmed the significant association of previously known BMD loci: ESR1, TNFRSF11B, LRP5, SP7, ZBTB40, TNFSF11 and TNFRSF11A, but interestingly they identified several loci in regions not previously associated with BMD (Rivadeneira et al., 2009). Recently, variants in CATSPERB (Koller et al., 2010), MATN3, IGF1 (Li et al., 2011), SOD2 (Deng et al., 2011) and FONG (Kou et al., 2011) genes between many others, have been involved in BMD regulation and in the pathogenesis of osteoporosis. Evidences for genes or loci association with BMD are controversial in many cases (Ralston & Uterlinden, 2010). Further large scale studies will be necessary to address the role of gene variants on BMD and osteoporosis, but the importance of this studies lies in the potential uses and clinical implications since, besides of differences in the effect of variants, the identified genes might be important for drugs design to prevention and treatment of osteoporosis.

4. Association studies

During the last years, association studies among natural variations of our genome (gene polymorphisms) and particular phenotypic characteristics such as OP, have shown that the mechanisms that condition this heritable susceptibility are defined by the presence of mutations or polymorphisms in one or several genes that influence bone phenotype. In this case, it is important clarifying that the term polymorphism refers to the presence of two or more gene variants in the same allele, in such a way that the less common variant must have a frequency equal or higher on 1% of the population, otherwise, the variation is considered as a mutation. These changes in the normal sequence may involve several bases, as in case mini-satellites or VNTR (*variable number of tandem repeat*), where the size of repeated fragments range from 15 to 70 pairs of bases in tandem. Other kind of polymorphisms are of micro-satellite also known as STR (*short tandem repeats*), which characterize for showing variations in the nucleotide number (2-6 base pairs). Recently, single nucleotide variations also known as SNPs (*single nucleotide polymorphisms*) have been analyzed; in this case, the analysis of these variations represents a very commonly used tool in studies that intend associating certain allelic variants with phenotypic characteristics, specially the ones attributed to polygenic diseases (multi-factorial and complex). Table 2 shows several single nucleotide polymorphisms studied in relation to osteoporosis and bone mineral density. It can be observed that some polymorphisms have been consistently studied with respect to particular bone traits, such as BMD in specific anatomic regions and in some cases with fracture risk.

Polymorphisms in genes as ER α and β, IL6, VDR, Aromatase (CYP19), COL IA1, RANK and RANKL are between the most studied. There are several polymorphic sites which association with BMD or with osteoporosis has been demonstrated in many different

populations. The results in many cases have been controversial, for example the SNP G/A in ERα gene exon 8, have been associated with osteoporosis in Thailander (Ongphiphadhanakul et al., 2001) and in Mexican women (Gómez et al., 2007), but association was denied when it was studied in Spanish women (Riancho et al., 2006), in spite all three investigations were performed with posmenopausal women. The T/C SNP of ERα gene was associated with low BMD in Japanese women, but not in Afro-American, Caucasian or Chineese women and the A/G SNP of the same gene, was associated with low BMD only in Afro-American Women, but not in Caucasian, Chinese nor in Japanese women (Greendale et al., 2006). The differences between studies results might be due to the genetic background of studied populations, which emphasize the importance of performing studies to explore the polymorphisms in specific groups with the same characteristics to avoid the incorrect use of genetic markers. Differences between races were evident too in studies with the IL6 G572C polymorphism in which the results in Korean (Chung et al., 2003) and Japanese (Ota et al., 2001) populations were consistent associating the G allele with low BMD, meanwhile in the study performed with Caucasian US women (Ferrari et al., 2003), the G allele appears as a protective factor from bone resorption.

Discordances can certainly be seen due to the frequencies of some alleles in different populations. It is important to determine the frequency of the polymorphism in a general population study before to perform a case-control study, since some genetic sites could be not polymorphic in some populations or the variant might be present in very low frequencies and their analysis could give spurious or no association results. An example of a SNPs which could not be used as osteoporosis genetic markers in Korean population are the G174C and G/A polymorphisms in the promoter of the IL6 gene because they show a very low frequency of this polymorphisms which difficult to found associations (Chung et al., 2003). However, the same G174C SNP was analyzed in Caucasian American healthy women (Ferrari et al., 2003) and in Mexican osteoporotic and non osteoporotic women as well as in general population (Magaña, et al., 2008), obtaining that the C allele is a protective factor from bone resorption and from osteoporosis respectively. However, most of the VDR gene SNPs showed in table 2, were consistently associated with low BMD or with osteoporosis in a great variety of populations. SNPs in intron 10, exon 2 and promoter of the gene, have resulted associated in European (Bustamante et al., 2007b; Utterlinden et al., 2001) American (Kiel et al., 2007; Pérez et al., 2008; Moffet et al., 2007) and Asiatic (Mencej et al., 2009) populations and even in large scale studies with world´s population (Morrison, 2004). The colagen IA1 is one of the most studied genes involved in osteoporosis. Many SNPs have been consistently associated with BMD and osteoporosis in several populations in this gene. The G/T change has been associated with osteoporosis in almost all studied populations, for example in Mexican (Falcón-Ramírez et al., 20011) and in British (Stewart et al., 2006). Not all the polymorphisms have a functional effect on bone traits, but the presence of the polymorphism G/T in Sp1 site, alters the recognition of the Sp1 factor having effects on transcription, protein production and mechanical strength of bone.

The appropriate expression of the genes of the route of signaling RANK/RANK-L/OPG is essential in osteoclastogenesis process, and makes them some of the most investigated genes performing studies with specific allelic, genotypic and haplotypic variants in this genes searching for associations with bone mineral density. In this case, variations of a single nucleotide in the intron 1, 9, and others located in the 3´del region gene RANK have consistently shown their association with low bone mineral density in spine and hip in European populations (Paternoster et al., 2010; Styrkarsdottir et al., 2009, Xiong et al., 2006).

GEN	POLYMORPHISM	LOCATION	REFERENCES	OUTCOME
CALCR	C/T C/T	Exon 13 Intron 12	Xiong et al., 2006.	Associated with spine osteoporosis in European families
ER α	G/A	Exon 8	Ongphiphadhanakul et al., 2001. Riancho et al., 2006 Gómez et al., 2007.	Associated with osteoporosis in postmenopausal Thailander women. Not associated with BMD in postmenpausal Spanish women. Associated with spine osteoporosis in Mexican women.
	C/T	Intron 1	Ongphiphadhanakul t al., 1998. Wang et al., 2008.	Associated with high BMD of spine and radius in Thailander males. No association in Chinese of both genders.
	C/T	rs2234693	Greendale et al., 2006. Bustamante et al, 2007b	Low BMD in spine in Afro-American and Japanese women. Low BMD in femoral neck in Spanish women.
	C/G C/T	rs1884052 rs3778099	Kiel et al., 2007.	Associated with hip/spine osteoporosis, with bone mass and geometry in US families of European origin (Framingham study).
	C/T C/T	rs3020314 rs1884051	Wang et al., 2008.	Associated with hip fracture in Chinese population.
	T/C A/G	3' UTR rs728524	Greendale et al., 2006.	Low BMD in hip and/or spine in Japanese and Afro-American women, respectively.
	C/A C/G	rs726282 rs1801132	Limer et al., 2009.	Low BMD in European males.
ER β	G/C	Intron 3	Wang et al., 2008.	Associated with hip fracture in Chinese population.
	G/A	Intron 8	Massart et al., 2009.	AA and AC genotypes associated with hip fracture in Italian
	C/T C/A	Intron 2 Intron 8	Greendale et al., 2006.	Associated with low spine BMD in Caucasian and high hip BMD in Chinese women.
	T/C C/T	Intron 3 Intron 8	Rivadeneira et al., 2006.	Vertebral fracture risk in carriers of haplotype 1 (CC) in Dutch population.
	C/T	Intron 7	Shearman et al., 2004.	Low hip BMD in US population (Framingham study)
	T/C A/G	Promoter Promoter	Ichikawa et al., 2005.	Associated with spine BMD normal variations in US Caucasian men and women.
IL-6	G/C (G572C)	Promoter	Chung et al., 2003. Ota et al., 2001. Ferrari et al., 2003 Magaña et al., 2008.	C allele and increased BMD in premenopausal Korean women G allele associated with low BMD in Japanese postmenopausal women G allele as protective factor from bone resorption in healthy Caucasian US women older than 65 years.

GEN	POLYMORPHISM	LOCATION	REFERENCES	OUTCOME
IL-6	G/C (G174C)	Promoter	Chung et al., 2003.	No association with BMD of Korean premenopausal women, due to its low frequency among Korean population.
			Ferrari et al., 2003.	The C allele as a protective factor from bone resorption in healthy Caucasian US women older than 65 years.
			Magaña et al., 2008.	The C allele is associated as a protective factor in Mexican women.
	G/A	Promoter	Chung et al., 2003.	Korean premenopausal women. Not associated with BMD due to its low frequency among Korean population.
IL6R	C/T	Promoter	Bustamante et al., 2007a.	C/T and G/A polymorphisms associated with femoral BMD and body mass ratio; A/C associated with lumbar spine BMD. Spanish postmenopausal women.
	G/A	Promoter		
	A/C	Exon 9		
VDR	C/T	3' UTR	Kiel et al., 2007.	Associated with low BMD of femoral neck and spine in US population (Framingham).
			Bustamante et al., 2007b.	Associated with low BMD in Spanish postmenopausal women.
	A/C	Intron 10	Kiel et al., 2007.	Associated with low BMD femoral neck and spine in US population (Framingham).
			Morrison, 2004.	Associated with low BMD. World population.
	A/C	Intron 10	Bustamante et al., 2007b.	Associated with low BMD in Spanish postmenopausal women.
			Uitterlinden et al., 2001.	Not associated with BMD or fracture. Meta-analysis with world population.
	A/G	Intron 10	Kiel et al., 2007.	Associated with osteoporosis and BMD of femoral neck and spine in US population (Framingham).
			Bustamante et al., 2007b.	Associated with low BMD in Spanish postmenopausal women.
			Morrison, 2004.	Associated with osteoporosis. World population.
			Pérez et al., 2008.	Low BMD in spine and/or femoral neck in postmenopausal-menopausal Argentinean women.
	C/T	Exon 2	Bustamante et al., 2007b.	Associated with BMD; not clearly to osteoporosis in Spanish women.
			Pérez et al., 2008.	Low BMD in spine and/or femoral neck in postmenopausal-menopausal Argentinean women.
	A/C/G/T	Exon 2	Kiel et al., 2007.	Associated with osteoporosis and BMD of femoral neck and spine in US population (Framingham).
			Morrison, 2004.	Associated with osteoporosis. World population.
			Moffett et al., 2007.	C/C genotype Associated with low BMD in wrist and fracture risk in Caucasian postmenopausal US women.

GEN	POLYMORPHISM	LOCATION	REFERENCES	OUTCOME
VDR	C/T	Exon 2	Uitterlinden et al., 2001.	Associated with a larger number of fractures; it does not show significant differences as risk factor for osteoporosis in Dutch women.
	A/G	Promoter	Kiel et al., 2007.	Associated with osteoporosis and BMD of femoral neck and spine in US population (Framingham).
			Morrison, 2004.	Associated with osteoporosis. World population.
			Mencej et al., 2009.	Associated with osteoporosis in Slovenia women.
	A/G	Promoter region	Uitterlinden et al., 2001.	Associated with fractures but not with osteoporosis in Dutch women.
	A/C	rs2189480	Kiel et al., 2007.	Associated with low BMD of femoral neck and spine in US population (Framingham).
			Bustamante et al., 2007b.	Associated with low BMD of femoral neck and spine in postmenopausal Spanish women.
CYP19	ins/del TTC	Intron 4	Limer et al., 2009.	Low heel BMD with 1 or 2 copies of TTC in males of European countries.
			Riancho et al., 2005.	Low hip and spine BMD with TTC in Spanish women.
			Mendoza et al., 2006.	Low hip and spine BMD with TTC/G (rs10046) in Spanish women.
	T/C	Exon 3	Riancho et al., 2007.	Associated with higher vertebral fracture risk in Spanish women.
			Riancho et al., 2009.	Associated with low hip and spine BMD with TT genotype in Spanish women.
	C/T	3' UTR	Limer et al., 2009.	Low heel BMD in males of many European countries.
			Mendoza et al., 2006.	Low hip and spine BMD in Spanish women.
	C/G	5' UTR	Riancho et al., 2007.	Associated with vertebral fractures risk in Spanish women.
			Riancho et al., 2009.	Higher hip BMD with GG genotype in Spanish women.
	C/T	Exon I.6	Riancho et al., 2009.	Associated with high hip BMD with TT genotype in Spanish women.
	A/G	Between exons I.2 y I.6	Riancho et al., 2007.	Associated with vertebral fracture risk in Spanish women.
	C/G	3' UTR	Kiel et al., 2007.	Associated with osteoporosis and femoral neck BMD in US families of European origin (Framingham).
	G/A	Intron 2		
	C/T	3' UTR	Xiong et al., 2006.	Associated with hip/spine osteoporosis in US families.
	T/C	Intron 8	Xiong et al., 2006.	Associated with hip/spine osteoporosis. US/European origin families.
	C/T	Intron 2		

GEN	POLYMORPHISM	LOCATION	REFERENCES	OUTCOME
CYP19	T/C G/A C/T	Intron 3 Intron 4 Intron 5	Hong et al., 2007.	Associated with low (T/C) and high (G/A and C/T) BMD in Chinese men.
PTHR1	A/T C/T A/G T/C	Intron 1 Intron 2 Intron 8 Intron 10	Vilariño-Güell et al., 2007.	As haplotype, they are associated with total BMD, bone mass peak, and/or loss of BMD in spine and/or hip in European families (FAMOS), in Caucasian and British women (ALSPAC) .
OPG	G/A	3' UTR	Richards et al., 2008. Paternoster et al., 2010.	Associated with low BMD in spine in European population (Rotterdam study). Associated with low BMD cortical. Analyzed in women of the United Kingdom (ALSPAC) and Swedish men (GOOD).
	G/C	Exon 1	García-Unzueta et al., 2008. Kim et al., 2008. Lee et al., 2010.	High BMD with CC genotype in Spanish women. High BMD with CC genotype; dose effect of C allele in Korean women. Low spine BMD in European and Asiatic population.
	A/G	5' proximal region	Geng et al., 2007.	BMD high con AA genotype in Chinese women.
ITGA1	C/T T/G A/C	Exon 3 Intron 5 Intron 28	Lee et al., 2007.	Associated as alleles and also as haplotypes with hip osteoporosis in Korean women.
COLIA1	G/T	Intron 1	Stewart et al., 2006. Jin et al., 2009. Falcón-Ramírez et al., 2011.	Low BMD with haplotype -1997G/-1663 of lT/+1245T in hip and spine in British women. Low BMD and increment of fracture risk in hip, in British men and women. Associated with spine osteoporosis in Mexican women.
	G/T	Promoter	Stewart et al., 2006.	Low BMD in hip and spine of British women. BMD increment as haplotype with other SNPs of the gene, only in spine.
	Ins/del T	Promoter	Stewart et al., 2006. Jin et al., 2009.	Low BMD in hip and spine in British women. Low BMD and increment of hip fracture risk, in British men and women.
	C/A	Intron 11	Kiel et al., 2007.	Associated with the width of the femoral neck in US women.

GEN	POLYMORPHISM	LOCATION	REFERENCES	OUTCOME
RUNX2	A/T	Intron 3	Ermakov et al., 2006.	Associated with anthropometric femoral length in a study conducted in Israel.
	A/T	Intron 4		
	T/C	Intron 4		
	C/T	Promoter 2	Lee et al., 2009.	CC genotype shows a low BMD in postmenopausal Korean women in spine and hip.
	A/G	Exon 2	Vaughan et al., 2002.	The A allele was associated with an increment of BMD in Australian women.
Unknown gene	G/T	rs6696981	Styrkarsdottir et al., 2008.	Associated with hip and spine fractures in Australian, Icelandic and Danish women.
	A/G	rs7524102		
RANK		rs3018362	Paternoster et al., 2010.	Associated with low cortical BMD. Analyzed in women of the United Kingdom (ALSPAC) and Swedish men (GOOD).
			Styrkarsdottir et al., 2009.	Associated with low BMD in hip of Icelandic and European subjects.
	A/G	Intron 1	Xiong et al., 2006.	Analyzed as haplotypes, these 17 polymorphisms showed association with osteoporosis and decrease of BMD in hip and spine in European families.
	A/G	Intron 1		
	A/C	Intron 1		
	C/G	Intron 1		
	A/G	Intron 1		
	A/G	Intron 2		
	A/T	Intron 3		
	G/T	Intron 3		
	A/T	Intron 4		
	C/T	Intron 7		
	A/G	Intron 7		
	G/T	Intron 9		
	G/T	Intron 9		
	C/T	Intron 9		
	C/G	Intron 9		
	G/T	3' region		
	C/T	3' region		
	A/G	Intron 6	Koh et al., 2007.	Polymorphism associated with low BMD in ward´s triangle, trocanter and femur, in Korean population.

GEN	POLYMORPHISM	LOCATION	REFERENCES	OUTCOME
RANKL	C/T	Intron 1	Xiong et al., 2006.	Associated with hip BMD decrease in European origin families.
			Mencej et al., 2006.	CC genotype Associated with a low BMD in postmenopausal Slovenia women.
			Mencej et al., 2008.	Associated with low spine BMD in osteoporotic Slovenia women.
			Mencej et al., 2009	Associated with spine BMD decrease in postmenopausal Slovenia women.
	C/T	Intron 2	Xiong et al., 2006.	Associated with hip BMD decrease in European origin families.
	C/T	Intron 1	Mencej et al., 2006.	CC genotype associated with low hip and spine BMD of postmenopausal Slovenia women.
			Mencej et al., 2008.	Association to low spine BMD of postmenopausal Slovenia women.
	C/G	Intron 1	Mencej et al., 2008.	Associated with a BMD decrease in spine in postmenopausal Slovenia women.
	C/T	rs9594738	Styrkarsdottir et al., 2008.	Associated with low spine BMD and moderately associated with fractures, in Australian, Danish and Icelandic subjects.
	C/T	rs9594759		
HDC	C/T	3' region	Xiong et al., 2006.	Polymorphisms associated with hip osteoporosis in European origin families.
	A/C	3' region		
	A/C	5' region		
	C/T	5' region		
ADCY10	G/A	Exon 7	Ichikawa et al., 2009.	Positive association to spine BMD; modest effect on the BMD peak in spine of US population (sisters study).
	C/T	Intron 14	Ichikawa et al., 2009.	US men presented association to hip BMD and trend to association to spine BMD.
TWIST1	A/G	3' region	Hwang et al., 2010.	Associated with osteoporosis in postmenopausal Korean women.

Table 2. Gene polymorphisms associated with osteoporosis and bone mineral density.

Other variations of a single nucleotide in intron 1 of the RANK-L gene have repeatedly been associated with low BMD of hip and spine in European, Asiatic and European populations (Xiong et al., 2006; Mencej et al., 2006; Mencej et al., 2008; Styrkarsdottir et al., 2008). The presence of these polymorphisms on human genome, are relatively easy to identify since birth or even in prenatal stage. These polymorphisms show a well defined inheritance pattern and their distribution may show differences not only among family groups but among populations and ethnic groups. However, in this kind of studies, we must be extremely careful and constantly consider the potentially confusing effect of some variables, such as: heterogeneity of populations, caused by genetic admixture, specially product of population's migration, the number of individuals included in studies is very important as well as the proper selection of cases and controls, and finally, the method to analyze data (Spencer et al., 2009; Duncan et al., 2002; Macarty et al., 2008). Not considering these elements in association studies would easily led us to establish spurious associations (Koller et al., 2004). Defining the genetic basis of primary osteoporosis in any population is not a simple task, we face a multi-factorial and polygenic entity present in populations that may have a great genetic heterogeneity; however the exploration of bone structure and metabolism genetic control, would allow to know the molecular basis of diseases such as osteoporosis, which represents a new window to explore therapeutic opportunities that would facilitate management of bone disorders.

5. Epigenetics and osteoporosis

During the last years attempts have been made to analyze the relation between environmental and genetic factors in the so called "complex diseases" using epigenetic studies. Epigenetics studies causal interactions among “genes” and their “products” which give place to the “phenotype”, which represents the body manifestation of a specific genetic profile. Epigenetics analyzes hereditary changes in the gene expression without changes in the DNA sequence, thus representing an important nexus between genotype, environment and the presence of a disease (Dupont et al., 2009). In osteoporosis, as a polygenic entity in which environmental component plays a determinant role, several risk conditions of maternal origin as bad nutrition of the mother, particularly the lack of vitamin D, habits as smoking and exposition to chemical agents (possibly including some drugs that impact bone quality), have the capacity to induce hereditary changes on future generations, which may occur in very early stages of the embrionary development, even during the neonatal period and they can generate an “imprinting” in the pattern of gene expression; this pattern is hereditary and “semi-permanent” because epigenetic modifications are reversible (Jiang et al., 2004; Dupont et al., 2009). On the other hand, apparently there is a relationship between low weight and size at time of birth and a higher risk of osteoporotic fractures during adult stage. Then we should understand that besides genetic and environmental factors, “epigenetic” can influence genome expression, so the prevention of some maternal conditions represents a valuable opportunity to develop preventative strategies aimed to improve bone quality in future generations.

6. Conclusion

Increment in life expectancy in some populations, ageing, changes in life style, especially the ones related to nutrition quality and physical activity, plus the vertiginous technological

development are characteristics of modern civilizations. This fact generates without a doubt a glaring increment in the incidence of several chronic degenerative diseases which may become crippling as occurs with osteoporosis, where the complications directly or indirectly cause great social and economic costs; thereby, they represent a social and health services challenge. Considering environment effects on the bone phenotype and the modifications in life style of populations in present time, osteoporosis could be in the future a disorder that occurs in younger population, rather than preferentially in elder people. This situation could overpass the medical services answer capacity and the governmental budget assigned to the medical care and rehabilitation of these patients; so it is important to intensify the investigations leading to elucidate the physiopathology of this disorder and the most relevant processes in bone metabolism.

Genetic association studies enable identification of new genes related to bone metabolism. Knowledge of the function of its products will allow us attaining a better understanding of some aspects of bone metabolism not entirely explored yet and will open new opportunities for therapeutic development in osteoporosis. On the other hand, clinical research from which results association studies, makes possible to identify and associate genotypic profiles (haplotypes) of risk in families and populations and even in ethnic groups. There is no doubt that progress in this scientific knowledge field, technological progress and especially the various preventative strategies at different stages of life, including prenatal stage through the integral care of maternal health, will surely contribute to achieve a better understanding of the disease, a better care and especially a better prevention.

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3

Biomechanics of Osteoporosis: The Importance of Bone Resorption and Remodeling Processes

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1. Introduction

Bone is a vital, dynamic connective tissue that gives form to the body, supporting its weight, protecting vital organs, and facilitating locomotion by providing attachments for muscles to act as levers. It also acts as a reservoir for ions, especially for calcium and phosphate, the homeostasis of which is essential to life. These functions place serious requirements on the mechanical properties of bone, which should be stiff enough to support the body's weight and tough enough to prevent easy fracturing, as well as it should be able to be resorbed and/or formed depending on the mechanical and biological requirements of the body. Under normal physiological conditions, the structure/function relationships observed in bone, coupled with its role in maintaining mineral homeostasis, strongly suggest that it is an organ of optimum structural design. To fulfill these structure/function relationships adequately, bone is constantly being broken down and rebuilt in a process called remodeling. Bone has the potential to adapt its architecture, shape, and mechanical properties via a continuous process termed adaptation in response to altered loading conditions (Burr et al., 2002; Forwood & Turner, 1995; Hsieh & Turner, 2001). Under normal states of bone homeostasis, the remodeling activities in bone serve to remove bone mass where the mechanical demands of the skeleton are low, and form bone at those sites where mechanical loads are transmitted sufficiently and repeatedly. An early hypothesis about the dependence of the structure and form of bones, and the mechanical loads they carry, was proposed by Galileo in 1638 (Ascenzi, 1993), and was first described in a semiquantitative manner by Wolff (Wolff, 1892). The adaptive response of bone has been a subject of research for more than a century and many researchers have attempted to develop mathematical models for functional adaptation of bone.

In this chapter, a brief explanation about the bone structure and mechanics will be provided first. Then, the bone remodeling process and its relation with osteoporosis will be discussed. The important issue of bone quality makes another section of this chapter.

Two mixture models of bone resorption, a bi- and a tri-phasic model of bone resorption will be reviewed, followed by a 2D model investigating the effects of osteocytes number and mechanosensitivity on bone loss. Discussion and conclusions make the last section of this chapter.

2. Bone structure and mechanics

Bone is the main constituent of the skeletal system and differs from the connective tissues in rigidity and hardness. The rigidity and hardness of bone enable the skeleton to maintain the shape of the body; to protect the vital organs; to supply the framework for the bone marrow; and also to transmit the force of muscular contraction from one part to another during movement. It is made basically of the fibrous protein collagen, impregnated with a mineral closely resembling calcium phosphate (Currey, 2002). The mineral content of bone acts as a reservoir for ions, particularly calcium (almost 99% of the calcium of our body is stored in bone), and it also contributes to the regulation of extracellular fluid composition. It also contains water, which is very important mechanically, some not well understood proteins and polysaccharides, living cells and blood vessels. The organic matrix of bone consists of 90% collagen, the most abundant protein in the body, and about 10% of various noncollagenous proteins (Behari, 1991). The protein part, mainly collagen type I, forms a model for the subsequent deposition of hydroxyapatite, the mineral phase of bone which provides rigidity to the structure. From mechanical point of view, bone is a nonhomogeneous and anisotropic material. Spongy and cortical bones can be considered as orthotropic and transversely isotropic materials, respectively. In the physiological range of loading, bone can be assumed as a linear elastic material, with negligible viscoelastic effects (Rouhi, 2006a). Bone is stronger in compression than in tension, and much greater young's moduli of elasticity than shear modulus (Bartel et al., 2006).

Outstanding mechanical properties of bone can be achieved by a very complex hierarchical structure of bone tissue, which has been explained in a number of reviews (Weiner and Wagner, 1998; Fratzl et al., 2004; Fratzl and Weinkamer, 2007). The mechanical performance of bone tissue depends on all levels of hierarchy. The term composite is usually employed for those materials in which two or more distinct phases are separated on a scale larger than the atomic, and in which their material properties such as stiffness and strength are altered compared with those of a homogeneous material. On the basis of the definition of a composite and also by considering bone structure, it is clear that bone is a composite material. Bone, as a biocomposite, shows hierarchical structures at different scales (Lakes, 1993). For example, in cortical bone, on the microstructural level, there are osteons or Haversian systems, which are large hollow fibers (200 to 250 μm outer diameter) composed of concentric lamellae and of pores. The lamellae are made up of fibers, and the fibers contain fibrils.

At the molecular level, the sophisticated structural interaction between the organic and inorganic phases is one of the fundamental determinants of the astonishing mechanical properties of bone. The underlying assumption is that a strong bonding between mineral and collagen allows the former to stiffen the collagen matrix through shear stress transfer. There are some important questions related to the composite nature of bone, which need to be addressed in order to make one able to understand the mechanics of bone as a composite at different hierarchical levels, such as: What are the properties of organic and mineral

phases of bone?; How do the organic and the inorganic phases of bone interact to offer the superior mechanical properties?; How do the cross-links within and between collagen fibrils contribute to the mechanical properties of the collagen?; and How is load and stress distributed between the collagen and mineral?. It is known that improperly mineralized tissues are often resulted when there is flaw in organic phase of bone for mineral deposition (Lucchinetti, 2001). At the nanoscale, bone is a composite of a collagen-rich organic matrix and mineral nanoparticles made from carbonated hydroxyapatite. The basic building block of the bone material is a mineralized collagen fibril of between 50 and 200 nm diameter. The collagen fibrils are filled and coated by mineral crystallites (Rubin et al., 2004); the latter are mainly flat plates that are mostly distributed parallel to each other in a fibril, and parallel to the long axis of the collagen fibrils (Landis, 1996). These well organized structural features have been associated with various unique structural properties of bone. For instance, the stiffness of bone is related to the composite structure of mineral micro-crystals and collagen fibers (Lakes & Saha, 1979); and the cement lines as weak interfaces convey a degree of toughness to bone (Piekarski, 1970).

Bone is a porous structure with different values of porosity depending on its macrostructure. At the macroscopic level, there are basically two types of bone structures: cortical (compact or Haversian) and cancellous (spongy, or trabecular) bone. Cortical bone is a dense, solid mass with only microscopic channels, and with a maximal density of about 1.8 gr/cm3. Approximately 80% of the skeletal mass in the adult human is cortical bone, which forms the outer wall of all bones and is largely responsible for the supportive and protective function of the skeleton. The main structural unit of the cortical bone is called osteon, or a Haversian system (Rouhi, 2006a). A typical osteon is a hollow cylinder with the outer and inner diameters of about 200 (or 250) and 50 μm, respectively. An osteon is made up of 20 to 30 concentric lamellae, and surrounding the outer border of each osteon there is a cement line, a 1-2 μm thick layer of mineralized matrix deficient in collagen fibers, which it is believed they act as crack stoppers when cracks are present. On the other hand, cancellous (spongy or trabecular) bone is a lattice of narrow rods and plates (70 to 200 μm in thickness) of calcified bone tissue called trabeculae, with an average thickness of 100-150 μm (Van der Meulen & Prendergast, 2000). The trabeculae are surrounded by bone marrow that is vascular and provides nutrients and waste disposal for the bone cells. The symmetry of structure in cancellous bone depends upon the direction of applied loads. If the stress pattern in spongy bone is complex, then the structure of the network of trabeculae is also complex and highly asymmetric. Comparison of micrograph structures with the density maps show that low density, open cell, rod like structure develops in regions of low stress while greater density, closed cell, plate like structures appear in regions of higher density in cancellous bone (Gibson, 1985). There are no blood vessels within the trabeculae, but there are vessels immediately adjacent to the tissue. Trabecualr bone is less mineralized than cortical bone, and experimental evidence and data suggest that spongy bone is much more active in remodeling than that of cortical bone (Guo & Goldstein, 1997). With ageing there are changes in the microarchitecture of bone. There is thinning of the cortex and of trabeculae, and a loss of connectivity, in particular of the horizontal trabeculae. The major cellular elements of bone include osteoclasts (bone resorbing cells), osteoblasts (bone making cells), osteocytes (bone sensor cells) and bone lining cells (inactive cells on the resting surfaces of bone) (Burger & Klein-Nulend, 1999). While osteoblasts and osteoclasts have

opposite functions and have different developmental origins, they exhibit several parallel features, particularly with respect to their life cycles. Osteoblasts and osteoclasts are both temporary cells with relatively short life spans (Parfitt, 1995).

3. Bone remodeling process and osteoporosis

During growth, bone is formed in the necessary places and resorbed as needed to attain the final shape, in a process called modeling. Modeling involves resorption drifts and formation drifts that remove or add bone over wide regions of bone surfaces. Thus, in modeling, bone resorbing and making cells act independently and at different spots. Modeling controls the growth, shape, size, strength, and anatomy of bones and joints. Collectively, modeling leads to increasing the outside cortex and marrow cavity diameters, shaping the ends of long bones. Modeling allows not only the development of normal architecture during growth, but also the modulation of this architecture and mass when the mechanical condition changes. When bone strains exceed a modeling threshold window, the minimum effective strain, modeling in the formation mode is turned on to increase bone mass and strength, and lower its strains toward the bottom of the window. When strains remain below the modeling threshold, mechanically controlled formation drifts stay inactive. As the forces on bone increases 20 times in size between birth and maturity, modeling in the formation mode keeps making bones strong enough to keep their strains from exceeding the modeling threshold, and therefore from reaching the microdamage threshold (Jee, 2001). In the adult age, the localized and independent activities of cells in modeling, are replaced by a distributed and coordinated work of the cells, resulting in a dynamic state called remodeling process. The actual remodeling occurs in two steps: the osteoclasts attach to the bone surface, dissolve the mineral, and later the organic phase of the bone, opening a hole that is subsequently filled by a number of osteoblasts, which produce the collagen matrix and secrete a protein which stimulates the calcium phosphate deposition. In the bone remdoeling process, resorption of extra-cellular matrices by osteoclasts (Teitelbaum & Ross 2003) is followed by osteoblastic invasion of the cavity, and subsequent secretion of extra-cellular matrix that is then mineralized (Ducy et al. 2000). These two processes, which together are called bone remodeling, occur continuously from birth to death and are in balance in a healthy bone (Riggs et al. 2002). This state can be shifted in favour of bone formation or resorption by mechanical stimulation, hormonal effects, nutrition, or diseases among other factors (Rouhi, 2006). Optimal remodeling is responsible for bone health and strength throughout life. An imbalance in bone remodeling may cause diseases such as osteoporosis. Bone remodeling occurs throughout life in thousands of sites within the human skeleton. The cellular link between bone resorbing cells or osteoclasts, and bone forming cells or osteoblasts, is known as coupling. How bone resorption and bone formation are linked is not entirely understood, but the consequences of accentuating one or the other preferentially leads to disease.

It was postulated that bone remodeling occurs to repair microdamage in bone (Frost, 1985; Mori & Burr, 1993). It was suggested that disruption of the canlicular connections occur when microcracks cut across them and can provide the stimulus to launch remodeling. It is well accepted that an unharmed gap junction intercellular communication or osteocyte-canalicular system inhibits the activation of osteoclast resorption and that interruption of the connection, for instance osteocyte apoptosis or microdamage, prevent the inhibition signals

to osteoclasts and so bone resorption starts (Martin, 2000). Gradual and diffusive osteocyte death has been reported with aging that can lead to enhanced bone remodeling and bone loss. Moreover, osteocyte death can make bones more brittle and vulnerable to fatigue damage, and bone remodeling bone loss (Jee, 2001). There are several reasons for the necessity of remodeling process, for examples: immature bone formed at the metaphyses is structurally inferior to mature bone; or the quality of adult bone deteriorates with time; or microcracks produced in bone by daily activity should be removed to attain a desired strength in bone; and/or ions concentration (e.g. calcium) should be adjusted to lie in an acceptable range; and, most likely, other factors that will be known in the future (Rouhi, 2006a). Assuming normal rates of adult bone remodeling, cortical bone has a mean age of 20 years and cancellous bone 1 to 4 years (Parfitt, 1983). Numerous theories related to the bone remodeling process have been proposed so far (see for instance, (Cowin & Hegedus, 1976; Hegedus & Cowin, 1976; Beaupre et al., 1990; Mullender et al., 1994; Jacobs et al., 1997; Rouhi et al., 2004 & 2006b))

Many diseases are related to global shift in the bone remodeling balance, for example: Osteoporosis, which is caused by increased osteoclast activity; Osteopetrosis, which is an abnormal increase in bone density by reduced osteoclast activity, Osteopenia, which is the bone loss by decreased osteoblast activity. The balance between bone resorption and bone formation is maintained through a complex regulatory system of systemic local factors acting on bone cells, such as calcium regulating factors, sex hormones, growth factors, and cytokine. The signal responsible for termination of bone resorption and initiation of bone formation are not well understood; however, evidence suggests that liberation of matrix embedded insulin- like growth factor system components may induce the shift. During bone turnover, surplus products synthesized by the osteoblasts during bone formation or fragments released during bone resorption are found in blood and urine. Too much bone resorption at the expense of formation results in osteoporosis, a loss of bone strength and integrity, resulting in fractures after minimal trauma. This leads to a disturbance in the bone's microarchitecture, which increases the probability of fractures. Osteoporosis is often called a "silent disease" because there are no symptoms until a bone breaks. Osteoporosis is a condition characterized by low bone mineral density and microstructural deterioration of bone tissue, leading to enhanced bone fragility and structural failure of the skeleton under low loads. Osteoporosis is a disease of enormous socioeconomic impact that is characterized by increase bone fragility (Seeman and Delmas, 2006). Such fragility is generally associated with an abnormal loss in bone volume, deterioration in the quality of the bone microarchitecture, an increased bone turnover rate, and also a shift of bone mineral density towards a lower mineralization density. Bone fragility can be defined from the pathophysiological point of view as "...the consequence of a stochastic process, that is, multiple genetic, physical, hormonal and nutritional factors acting alone or in concert to diminish skeletal integrity (Marcus, 1996)". The treatment of the bone diseases is based on drugs that intend to restore the remodeling equilibrium. Most of the work on osteoporosis, probably the most important of these diseases, seems to be currently in the osteoclast inhibition side (Rodan & Martin, 2000; Teitelbaum, 2000; Rouhi et al., 2007).

Peak bone mass (PBM) corresponds to the amount of bony tissue present at the end of skeletal maturation. It is a major determinant of the risk of fracture later in life, because there is an inverse relationship between fracture risk and areal bone mineral density, in

women, as well as in men. Interaction between genetic and non-genetic factors on bone mineral mass and structure changes during puberty. Genetic factors are either acting directly on bone or indirectly by modulating the sensitivity to environmental factors. Similarly, environmental factors are acting either directly on bone or indirectly by modulating the genetic potential. Human bone mass increases during growth, levels off in young adult life, and after about 30 years it starts to decrease. The most common sites of bone fracture are spine, hip, and wrist. The main cause of osteoporosis is the continuous loss of bone during life, which is intensified in female after menopause and male with andropause. At age 70 years, 70% of the young adult mass can remain (Wanich, 1999). It is known that with ageing, bone is lost from all parts of the skeleton, but not in equal amounts. Another factor is a lesser bone production during maturation, which cause a reduction in peak bone mass. Both cortical and cancellous bones are primarily thinned by the removal of bone at the endosteal surfaces adjacent to bone marrow. Cortical bone loss occurs mostly at the cortical endosteal surface and to a small degree from the increase in the radius of the Haversian canals. A small net gain of bone partly offsets this lost at the periosteal surface (Martin and Burr, 1989; Frost, 1999a). Age-related cancellous bone loss is because of the imbalance in bone remodeling with excessive bone resorption relative to bone formation. The sequence of Activation-Resorption-Formation is often uncoupled because of reducing the available trabecular rods/plates surfaces for bone formation. In elderly people, the most common cause of increased bone resorption is calcium and vitamin D deficiency, which will result in secondary hyperparathyroidism. Muscle mass and strength increases during growth and plateaus in young adults and then declines. Interesting to know that muscles apply the largest loads on bone, and bones normally adapt their mass and strength to the largest load. Thus, age-related reduction in muscle mass and strength can be deemed as a major factor for the age-related reduction in bone apparent density and strength (Bucwalter, et al., 1993; Frost, 1999 a&b; Burr, 1997). Needless to emphasize that loss of muscle mass and strength will increase the tendency to fall, and thus will increase the fracture risk.

4. Factors determining bone quality

The quality of bone tissue relates to its composition and microstructure, whereas its quality as an organ depends also on its macrostructure. The strength of a bone and its ability to perform these physical functions depend on its structure and the intrinsic properties of the materials of which it is composed. The amount of bone, its spatial arrangement, its composition, and its turnover are all determinants of its ability to perform mechanical functions and to resist fracture. Bone quality is determined by at least four factors as follows: Properties of the organic and mineral phases of bone, also the collagen-HAp composite structure; Microdamage accumulation; Architecture and geometry of cancellous and cortical bone; and finally Rate of bone turnover and remodeling. Organic and mineral phases, i.e. collagen and hydroxyapatite, and architecture changes with age, bone diseases, such as osteoporosis, and therapeutic treatment. The risk of fracture in a 75-year-old woman can be 4-7 times that of a 45-yr-old woman with identical bone mass, demonstrating a bone quality component of fragility that is independent of bone mass.

The fracture resistance of bone results from the ability of its microstructure to dissipate deformation energy, without the propagation of large cracks leading to eventual material failure (Currey, 1999; Currey, 2003; Taylor et al., 2007). One striking feature of the fracture

properties in compact bone is the anisotropy of the fracture toughness, which differs by almost two orders of magnitude between a crack that propagates parallel or perpendicular to the fibril direction. This dependence of fracture properties on collagen orientation underlines the general importance of the organic matrix and its organization for bone toughness (Seeman & Delmas, 2006). Mechanical properties of bone are determined by a number of structural features, including: the mineral concentration inside the organic matrix; the size and mechanical properties of mineral particles; the quality of the collagen, in term of its amino-acid sequence, crosslinks and hydration; the quality and composition of the extrafibrillar organic matrix between the collagen fibrils; and the orientation distribution of the mineralized collagen fibrils. The mineral concentration inside the organic bone matrix is a major determinant of bone stiffness and strength (Seeman & Delmas, 2006; Currey, 2001; Currey, 2002). However, the mineral content within both the trabecular and the cortical bone is far from homogeneous. At least two processes that occur in bones over the whole lifetime of an adult individual are responsible for this situation: bone remodeling and kinetics of matrix mineralization. The newly formed bone matrix is initially unmineralized (osteoid), but after an initial maturation time of about 2 weeks, the bone goes through a stage of rapid mineralization, where 70% of the full matrix mineral content is achieved in a few days (primary mineralization). Then, the mineral content increases very slowly to reach full mineralization within years (secondary mineralization) (Boivin & Meunier, 2003).

Fracture risk increases with age, partly as a function of changes in bone mineral density. Aging is associated with a reduction in collagen content. In osteoporosis, there is an increase in both synthesis and degradation of collagen, and an increase in the number of immature cross-links. Osteoporotic bone may be more fragile due to fewer collagen fibers and weaker cross-linking. Questions such as: How do therapeutic treatments for osteoporosis alter collagen quality (contents, cross linking, turnover rate)?; and How does increased bone turnover affect collagen quality?, are still open and need to be addressed in the future. Although changes in bone mineral content are widely recognized to occur in aging and osteoporosis, the physicochemical properties of the mineral crystal may also be changed. Mineral crystallinity increases with age, and this in itself may make the tissue more brittle. Anti-resorptive therapies increase tissue mineralization by increasing the mean tissue age. Whether this is beneficial or deleterious is not clear yet. However, the increase in mineralization never achieves the level of mineral in normal non-osteoporotic age-matched controls, so it is likely to be a positive change. However, anti-resorptive therapies also have a tendency to make the tissue mineralization more uniform, from a fracture mechanics point of view, and this would make it more likely for cracks that are introduced into the matrix to grow. There are still many questions in regard to the mineral phase of bone, such as: How is bone crystallinity affected by long-term antiresorptive therapies?; What role do osteocytes play in matrix mineralization?; What is the relationship between mineral crystallinity and brittleness?; What is the mechanical effect of reduced variability in bone mineral distribution (i.e. increasing homogeneity of tissue properties)?, which need to be addressed in the future. Structural changes, some of which are independent of bone mass, also occur in osteoporosis. In osteoporosis, there is a tendency to convert to a more rod-like and more anisotropic structure, whereas bisphosphonate treatments tend to make the bone more plate-like and more isotropic.

Complete trabecular perforations increase as the remodeling rate increase. These may weaken the structure more than expected based on the loss of bone mass alone. Regarding the effects of bone architecture on its quality and mechanical properties, there are some questions such as: Does maintenance of anisotropy reduce bone fracture risk?; To what extent do resorption bays in trabeculae waken bone?; and What is the relative role of trabecular and cortical bone in vertebral and hip fracture risk?, which need to be answered.

A reduction in fracture toughness of bone with age was reported in the literature, which was caused either because of an increase in mineralization (Currey et al., 1996; Zioupos et al., 1998) or alterations in the collagen matrix (Zioupos et al., 1999). In an animal model of disuse osteoporosis, a reduction in collagen cross-links can be seen (Yamauchi et al., 1988). Other experimental evidence supports the idea that the concentration of collagen cross-links is considerably lower in osteoporotic individuals compared to age-matched controls (Oxlund et al., 1996). It should be noted that the initial cross-links between collagen molecules are unstable, but as bone matures, the cross-links also mature into more stable nonreducible forms. So, there is an increase in collagen matrix's density, stiffness, and strength during maturation (Bailey & Paul, 1999). It should also be noted that the content of mature cross-links is lower in cancellous bone as compared to cortical bone, due to the greater rate of the cancellous bone remodeling (Eyre et al., 1988). The bone collagen cross-links are usually modified in the mineralization process.

5. A bi-phasic mixture model of bone resorption process

Osteoporosis, regardless of etiology, always represents enhanced bone resorption relative to formation. Thus, insights into the pathogenesis of this disease, and progress in its prevention and/or cure, depend on understanding the mechanisms by which bone is degraded. The osteoclast is the principal resorptive cell of bone, and the most successful treatments of osteoporosis, to date, target osteoclastic bone resorption. The osteoclast is a multinucleated cell whose capacity to degrade hard tissues, among other factors, depends on cell/matrix contact. All forms of adult osteoporosis reflect enhanced bone resorption relative to formation, and should be viewed in the context of the remodeling cycle. The reason for using this way of treatment is the lack of information about all various factors affecting osteoclasts' activity. Biological tissues, including bones, are all composed of multiphase constituents, and there are chemical reactions and/or diffusions between different components of them. Cells, as live organs in the biological tissues, can dictate rate of growth and adaptation, and their activities are affected by different, including mechanical, chemical, and biological factors.

Here a brief explanation about a recently proposed biphasic mixture model of bone resorption is presented (Rouhi et al., 2007). This model aims at shedding some light on the bone resorption process using a multi-constituents continuum mechanics model. In this model, bone is treated as a biphasic mixture of matrix and fluid, and bone resorption is considered as an exchange of mass between the solid and fluid phases. This exchange is caused by the secretion of H^+ and Cl^- from osteoclasts, which creates an acidic environment in a sealed microenvironment between the osteoclasts and the bone matrix (Blair 1998; Rousselle and Heymann 2002). The governing equations for bone resorption can be derived using the conservation laws, entropy inequality, and the appropriate constitutive equations.

In the conservation of mass equations, the rate of mass transferred to different constituents is assumed to be given by an empirical relation arising from the dissolution kinetics of the solid phase. In the constitutive equations, it is assumed that dependent variables, such as free energy, are a function of temperature, deformation gradient, rate of deformation gradient, and the extent of chemical reactions (Rouhi et al., 2007).

It should be noted that bone mineral (hydroxyapatite) and organic (collagen I) matrix are degraded independently. Thus, a bone resorption model needs two separate expressions, one because of the each phase. Because of the lack of information about the dissolution of the organic phase, we only considered the mineral phase dissolution and assumed that it is equivalent to the dissolution of the bone matrix. Microscopic observations suggest that degradation of collagen closely follows mineral degradation (Chambers et al. 1984), so our assumption may be justified. Dissolution of minerals occurs at the bone surface. A major source of uncertainty is the surface reactivity, which depends on chemical composition, atomic structure, and surface topography. The free energy of surface sites changes as a function of the aforementioned factors. Thus, no universal expression for the dissolution kinetics exists and experimental studies are needed to derive a dissolution kinetics relation for each case. The dissolution kinetics of hydroxyapatite has been the subject of numerous studies so far (Christoffersen et al. 1996; Dorozhkin 1997a; 1997b; 1997c; Thomann et al. 1989; 1990; 1991; Margolis and Moreno 1992; Hankermeyer et al. 2002; Fulmer et al. 2002; Chow et al. 2003). Because of the small dimensions of the resorption microenvironment between the osteoclasts and the bone matrix assuming that dissolution is governed by the reaction kinetics seems logical and acceptable.

In order to develop a general framework for the description of bio-chemo-mechanically driven bone resorption, some basic assumptions should be made as follows: Bone is a biphasic mixture of a solid phase and a fluid phase; The transfer of mass, energy and entropy between the solid and the fluid phases are a result of biochemical reactions that occur between the osteoclasts and the matrix; The characteristic time of chemical reactions is several orders of magnitude greater than the characteristic time associated with a complete perfusion of the blood plasma in bone, so the resorption process can be considered isothermal; The bone matrix is isotropic and linearly elastic; Mechanical, chemical, and biological factors affect the rate of bone resorption, thus they all appear in the bio-chemo-mechanical affinity as the driving forces of the chemical reactions; and finally Dissolution of the matrix is the same as resorption of the mineral phase. Furthermore, it is assumed that the degree of saturation is a function of the bio-chemo-mechanical affinity, but not just of the Gibbs free energy.

Bone resorption can be simplified to (see (Blair 1998; Dorozhkin 1997a; 1997b; 1997c)):

$$Ca_{10}(PO_4)_6(OH)_2 + 2H^+ \longrightarrow 10Ca^{2+} + 6PO_4^{-3} + 2H_2O \quad (1)$$

The chemical driving force for bone resorption, i.e., the chemical reaction shown in Equation (1), can be expressed by the Gibbs free energy variation per mole. In 1992, Margolis and Moreno (Margolis & Moreno, 1992) performed dissolution experiments with hydroxyapatite crystals, in which they measured pH, calcium and phosphate concentrations at a constant temperature. They proposed the following equation for the rate of dissolution of the mineral phase of the bone matrix:

$$J = k(1 - DS)^m [H^+]^n \quad (2)$$

where J is the mineral flux across the real surface of the mineral phase, DS is the degree of saturation, $[H^+]$ is the concentration of hydrogen ion, and k, m, and n are empirical constants. As stated earlier, it is assumed that the mineral flux, J, is almost the same as the dissolution rate of the solid phase, i.e. hydroxyapatite + collagen fibers.
The degree of saturation (DS) is expressed as:

$$DS = \{([Ca^{2+}]^5 [PO_4^{-3}]^3 [OH^-])/Kso\}^{1/9} \quad (3)$$

where [X] is the concentration of ion X, and Kso is the solubility product of hydroxyapatite (Margolis and Moreno 1992).
Since biological, chemical, and mechanical factors have a definite effect on the rate of dissolution, it is hypothesized that a bio-chemo-mechanical driving force should be considered in the dissolution relation, instead of just a chemical driving force (i.e. just changes in the Gibbs free energy). Dissipation law can be used to find the bio-chemo-mechanical affinity, and it is defined as the difference between the external work rate and the rate of change in free energy. According to the Second Law of Thermodynamics, this quantity should be nonnegative. Using the dissipation law and after some manipulations, the driving force for the dissolution process of bone can take the following form:

$$A = \psi_{mech.} + P + C_s (\mu_s - \mu_{ext}) \quad (4)$$

where $\psi_{mech.}$ is the mechanical part of the free energy, P is the hydrostatic pressure, C_s is defined as ρ/M, where ρ and M are the density and the molar mass of the matrix, respectively, and μ_s & μ_{ext} are the chemical potential of the solid phase in the unstressed condition and the external potential energy, respectively.
Our bi-phasci mixture model of bone resorption shows that the activity of osteoclasts and, thus, the rate of bone resorption are not only dictated by biological factors (e.g., hormone levels), but also by engineering quantities, i.e. hydrostatic pressure, strain energy density, and concentration of different ions before and after the resorption process. Interesting to note that the exact stimulus for the initiation of the remodeling process of bone is not known yet and is a place of debate (Rouhi, 2006a). In 1990, Brown and co-workers have shown experimentally that strain energy density can be a likely stimulus for bone remodeling (Brown et al. 1990), and it was used extensively in many theoretical modeling of bone adaptation; for instance (Jacobs et al. 1997; Huiskes et al. 2000; Doblar´e and Garc´ıa 2001; Garcia et al. 2002; Ruimerman et al. 2005). As can be seen in Eq. 4, in this bi-phasic model, strain energy density is appeared as an effective mechanical stimulus for the bone resorption. Moreover, using our bi-phasic model, hydrostatic pressure was introduced as another mechanical stimulus for the bone resorption process (see Eq. (4)). Using this model, it was also shown that increasing either strain energy density or hydrostatic pressure will increase rate of bone resorption. The former point can be used as a theoretical justification for many experimental observations (e.g., (Burr et al. 1985; Burr and Martin 1993; Mori and Burr 1993; Schaffler and Jepsen 2000; Li et al. 2001; Martin 2003; Van Der Vis et al. 1998; Skripitz and Aspenberg 2000; Astrand et al. 2003). This model also shows that an increase in the concentration of H^+, or a decrease in the concentrations of PO_4^{-3} and Ca^{2+} can cause a reduction in the rate of bone resorption. Experimental data can be found in support of this model's predictions of the effect of Ca^{2+} concentration on the rate of bone resorption (Lorget et al. 2000). Using the Second Law of

Thermodynamics, it was also shown that the maximum rate of bone resorption in cortical bone is greater than that of cancellous bone. This behaviour of cortical and trabecular bone, which is well accepted experimentally (Martin & Burr, 1989), can also be predicted using the axiom of mass balance in this bi-phasic model.
For more detailed information about the basic assumptions, also governing equations of the bi-phasic model of bone resorption, interested readers are encouraged to consult the following reference (Rouhi et al., 2007).

6. A tri-phasic mixture model of bone resorption process

Recently, a tri-phasic model of bone resorption using mixture theory with chemical reactions was proposed (Rouhi, 2011). In this model, three different constituents (matrix, fluid, and cells) have been considered. Bone resorption is considered as a chemical reaction caused by the secretion of H^+ and Cl^- from osteoclasts which creates an acidic environment in a sealed zone between osteoclasts and bone matrix. It is assumed that the solid phase obeys small deformation theory and is isotropic and linearly elastic. The velocity of the matrix and cells is assumed to be zero. The fluid phase is assumed to be viscous, and inertial effects are neglected because of the slow velocities that are at play. A non-rotational fluid is assumed for deriving the final form of the entropy inequality for the mixture as a whole. In the constitutive equations, similar to our bi-phasic model (Rouhi et al., 2007), it is assumed that the free energy, enthalpy, specific entropy, heat flux, and stress tensor are functions of temperature, deformation gradient, and the extent of chemical reactions. Bone resorption was considered as an isothermal and a quasi-static process. For the sake of simplicity, presence of ostocytes in the bone matrix was discarded in this model, despite the fact that fluid flow in the bone matrix (e.g. in the lacuno-canalicular network) has a definite effect on the osteocytes, and, most likely, on the osteoclasts and thus on the rate of bone resorption. Using these assumptions, the governing equations for bone resorption were derived using the conservation laws (mass, momentum, and energy), as well as entropy inequality and the appropriate constitutive equations.
By using mixture theory with chemical reactions, first, contribution of different phases present in the mixture can be observed. Secondly, using consistency requirement for energy balance, it was found that rate of bone resorption is a function of different factors including apparent density of bone matrix and bone fluid; fluid velocity; momentum supply to the fluid or solid phase; and internal energy densities of different constituents. Thirdly, using the relation between momentum supply to the solid and fluid phase, one can conclude that rate of bone resorption is inversely proportional to the bone fluid velocity. Also, it was found that in spongy bone, by increasing the porosity, rate of resorption will decrease and vice versa. Based on our results, it is speculated that bone resorption in cortical and cancellous bones might be affected by a control system which is resulted from the relation between the specific surface of bone and its apparent density and volume fraction. As it is known, one reason of osteoporosis is the lack of calcium ions in our body, so in the case of need of calcium, where is better than a rich reservoir, i.e. bones, to take away calcium ions via bone resorption process and giving back in the bone formation process. It seems necessary and feasible, as a future task, to investigate the relation between calcium concentration in the bone fluid and the rate of bone resotrpiton using mixture theory.

For more detailed information about the basic assumptions, also governing equations of the tri-phasic model of bone resorption, interested readers are encouraged to consult the following reference (Rouhi, 2011).

7. The effects of osteocytes number and mechanosensitivity on bone loss

Based on the experimental data and evidence, it is known that osteocyte density (the number of osteocytes per unit surface of bone) changes with aging and also in osteoporotic bones (Gong et al., 2008; Mullender et al., 1996). Moreover, they interestingly found that the osteocyte density increased in osteoporotic patients compared to that of healthy adults, although excessive bone loss and reduced spongy bone wall thickness have been described as characteristic for osteoporotic bones. Experimental evidence for altered mechanosensitivity of osteocytes derived from osteoporotic patients has also been reported (Sterck et al., 1998). According to the semi-mechanistic bone remodeling theory (Huiskes et al., 2000; Ruimerman et al., 2005), and based on the fact that the number of osteocytes per unit surface of bone decreases with aging, we hypothesized that bone loss with the age is correlated with the reduction of either the number of osteocytes, or the strength of the recruitment signal sent by osteocytes to osteoblasts (Li, 2011; Li & Rouhi, 2011).

In the semimechanistic model of Huiskes and co-workers, bone remodeling is considered as a coupling process of bone resorption and bone formation on the bone free surfaces. Osteoclasts are assumed to resorb bone stochastically. Osteocytes are suggested to act as strain energy density (SED) rate sensing cells, and to play a role in the regulation of bone remodeling. It is assumed that osteocytes locally sense the SED rate perturbation generated by either the external load or by cavities made by osteoclasts (bone resorbing cells), and then recruit osteoblasts to form bone tissue to fill the resorption cavities. Osteoclasts are assumed to resorb a constant amount of bone per day. The probability of osteoclast activities may be regulated by the presence of either micro-cracks or in the case of disuse. Since the changes in bone structure because of osteoporosis are similar to changes resulting from disuse (Frost, 1988; Rodan, 1991), it was assumed that one of the causes for bone loss in osteoporotic bones can be the reduction in osteocyte mechanosensitivity.

In our study, we developed a two dimensional finite element model of spongy bone using a semi-mechanistic bone remodeling theory (Huiskes et al., 2000) to simulate spongy bone remodeling and investigate the validity of our hypotheses (Li, 2011; Li & Rouhi, 2011). Results of our study showed that the osteocyte density has a significant role in the final geometry of spongy bone in the bone remodeling process. It was also shown that by decreasing the osteocyte density (knowing that the osteocyte density decrease as a healthy adult ages), bone loss will occur and there will be a decrease in bone apparent density. Moreover, it was shown that when osteocyte mechanosensitivity is less than a certain level, osteoporotic patients lose more spongy bone than healthy old adults even though osteoporotic patients have greater osteocyte number than in healthy old adults. Figure 1 shows the final simulation results of spongy bone with different mechanosensitivities of osteocytes, but the same osteocytes' number and the same form of osteocyte distribution. As can be seen, by decreasing the mechanosensitivity of osteocytes, there will be a reduction in spongy bone apparent density. Results of this study were in favour of our hypothesis stating that “by decreasing the osteocyte mechanosensitivity, as is the case in

an osteoporotic bone, bone apparent density will also decrease even by increasing the number of osteocytes".

Some of the possible explanations for the abnormal bone loss in an osteoporotic bone suggested by different researchers are as follows: (1) a higher percentage of the bone forming cells is embedded in bone matrix as osteocytes (Mullender et al., 1996), so a reduction in the number of bone forming cells can be seen; (2) the bone forming activity of osteoblasts is reduced (Mullender et al., 1996; Ruimerman et al., 200?), thus less bone apposition will occur; (3) the average life-span of osteoblasts is reduced (Mullender et al., 1996; Eriksen and Kassem, 1992); (4) a reduction in bone sensor cells mechanosensitivity (Sterck et al., 1998), thus they cannot make a true picture of the mechanical environment of the bone and so there will be a reduction in the smartness of bone structure. It seems reasonable to assume that bone loss in the case of osteoporosis is the result of a combination of all the above mentioned, and likely some other, factors.

For more detailed information about this work, interested readers are encouraged to consult the following references (Li & Rouhi, 2011; Li, 2011).

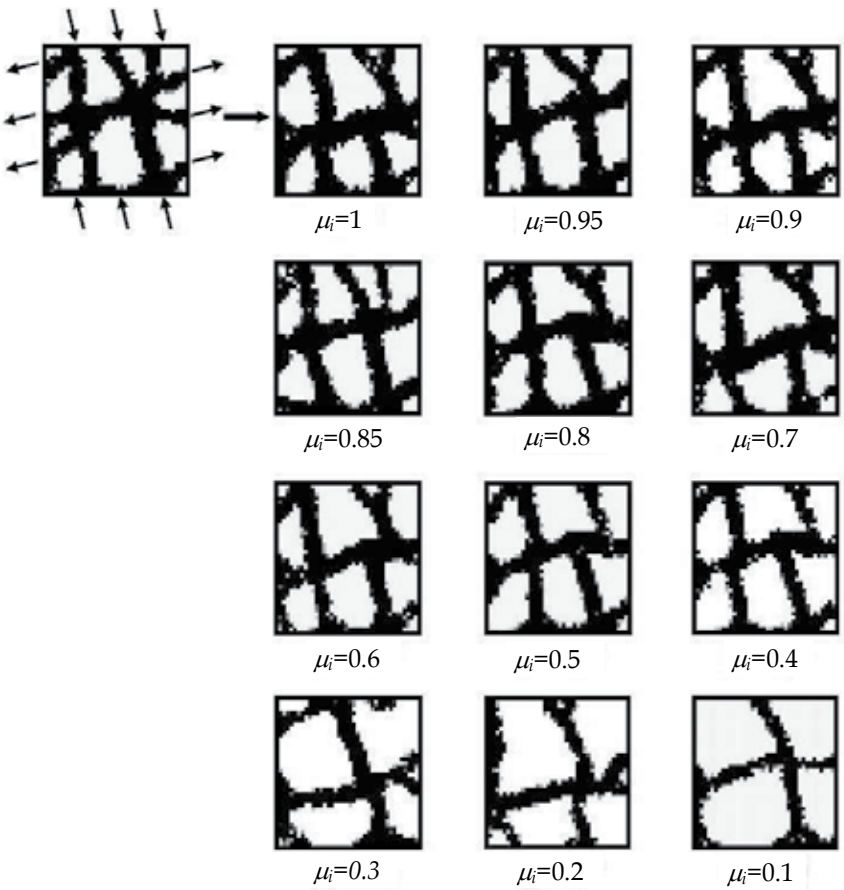

Fig. 1. Results of simulation of the spongy bone remodeling for different levels of osteocyte mechanosensitivity (μi), representing the level of activity of bone sensor cells (Li, 2011).

8. Discussion and conclusions

Unlike Engineering materials and structures, biological materials including bone, are sensitive to the mechanical stimuli placed on them. Moreover, their mechanical properties are changing continuously as a function of time, mechanical load, and biological factors (e.g. various hormones levels and nutrition). Osteoporosis is caused when there is an imbalance in the bone remodeling process. So, in order to be able to find a solid cure for this disease, a clear and comprehensive understanding of the bone remodeling process at different level of considerations, i.e. molecular; cellular; and tissue level, is needed. A wealth of evidence has been accumulated during the past few years supporting the concept that the study of bone micro- and nano-structures will not only improve our understanding of the mechanisms that underlie bone fragility, but also help to discover the effects of treatments. For instance, nanomedicine and its application to bone research can undoubtedly broaden our knowledge of patho-physiology and improve the diagnostic, prevention and treatment of bone diseases including osteoporosis. Considering the complexity and multifactorial aspect of the remodeling process, the best way to tackle this problem seems to be working in a multidisciplinary group including researchers from various disciplines of medicine and bioengineering.

Based on the fact that skeletal integrity is determined by the outstanding and variant mechanical properties of bone at different hierarchical levels of its structure, it becomes clear that a simple diagnostic parameter such as hip bone mineral density (BMD) does not have enough diagnostic strength to determine the complex patho-physiological mechanisms that determine bone fragility. Thus, new diagnostic tools developed by bioengineering scientists, coupled with a possible combinatorial approach using different methods to define the material qualities of bone at different hierarchical levels of bone's structure, are needed in identifying the initiation and also the progression of the silent and dangerous disease, so-called osteoporosis.

The responsiveness to either an increase or a decrease in mechanical stimulus is very likely greater in growing than adult bones. So, the concept of public health programs aimed at increasing physical activity among healthy children and adolescents in order to maximize peak bone mass, and thus to minimize the probability of bone fracture due t low strength, seems reasonable and should be considered seriously.

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Part 2

Public Health Data of Osteoporosis

4

Self-Reported Prevalence of Osteoporosis in Australia

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1. Introduction

"Self-report" is generally the only method of determining the prevalence of non-registry based chronic diseases (Bergmann et al., 2004). However, there are difficulties in "case definition" associated with self-report and often the most effective means of identifying the presence of disease is to determine whether the chronic condition in question has been diagnosed by the doctor. Chronic conditions such as osteoporosis are often difficult to identify as they do not generally manifest themselves until after a bone fracture occurs. The aim of this chapter is to determine the self-reported prevalence of osteoporosis and associated demographic factors from a community dwelling sample aged 15 years and over across a 16 year period and compare this prevalence with that obtained from a biomedical study. Associated risk and demographic factors can be examined using these data. The issues around the use of self-reported, doctor-diagnosed osteoporosis to determine disease prevalence will also be discussed.

2. Background

Osteoporosis is a hidden condition. Bone loss due to osteoporosis is subtle but as there are no overt symptoms, it is generally not until a fracture occurs that osteoporosis may be identified (Australian Institute of Health and Welfare [AIHW] 2011; Rachner et al., 2011; Sànchez-Riera et al., 2010). However, with an ageing population, the related medical issues and socioeconomic impact will only increase (Rachner et al., 2011). Osteoporosis is a condition which affects both men and women although the greatest focus has generally been on post menopausal women (Cawthon, 2011). A meta-analysis identified that there was a five to eight fold increase in the risk of mortality due to all causes within the first three months following a hip fracture (Haentjens et al., 2010), which is a common fracture type associated with osteoporosis (Cooper, 1997). An increased annual mortality remains over time and it is generally higher for men compared to women (Center et al., 2011; Haentjens et al., 2010). Fractures consequently are a significant health issue which lead to not only premature mortality but also an increased level of disability and risk of future fracture (Center et al., 2007; Center et al., 2011; Cooper, 1997).

Dual x-ray absorptiometry (DXA) is considered to be the gold standard for the diagnosis of osteoporosis (Keen, 2007). Guidelines have been developed and implemented to address effectively screening for osteoporosis (Rachner et al., 2011). In Australia, the guideline focuses on post menopausal women and older men (Royal Australian College of General Practitioners, 2010), as do other international guidelines (Compston et al., 2009; Hodgson et al., 2003). The guideline does not, however, come into effect until there has been a minimal trauma fracture (Royal Australian College of General Practitioners, 2010). Risk assessment tools have also been developed which combine clinical risk factors and DXA measurements (Borgström & Kanis, 2008; Unnanuntana et al., 2010). Thus DXA scans are only provided to those considered at risk of osteoporosis or in response to a minimal trauma fracture. Bone density screening is not provided to the population in a similar manner to breast cancer screening, as it is not considered cost effective, due to the cost of providing scans and limited availability (Davis et al., 2011).

A variety of data sources are used to determine the characteristics of osteoporosis within the Australian population and estimating the population prevalence can be difficult. Self–reported, doctor diagnosis of a condition is generally used in population surveys but these estimates do not generally reflect the true prevalence. This discrepancy with true prevalence has been demonstrated (Sacks et al., 2005) using arthritis information collected as part of the Behavioural Risk Factor Surveillance System (BRFSS) which is undertaken across the United States. In terms of osteoporosis, the prevalence is underestimated due to the absence of obvious symptoms until a diagnosis may occur following a minimal trauma fracture (AIHW, 2011; Werner, 2003). But even after a minimal trauma fracture, those with osteoporosis may be untreated or undiagnosed (Eisman et al., 2004) or the underlying disease may not be appropriately investigated (Elliot-Gibson et al., 2004).

Osteoporosis contributes to the global burden of disease. Chronic conditions (including osteoporosis), whether they are physical or mental, reduce quality living time, with the subsequent morbidity significantly impacting the population (McQueen, 2003). Regular surveillance allows the monitoring of health, demographic and other related data to assess trends and prevalence and also provide an explanation of demographic and exposure differences, the use of health services and evaluate if there is a response to health promotion and public health interventions (McQueen, 2003; Wilson, 2003). A system that monitors chronic disease and related risk factors does have some specific features which characterise it as "surveillance" (Campostrini, 2003). These include:

- Time, which is a essential element of the data collection,
- There is a focus on chronic or non-communicable diseases and related factors, and
- Attention is also focused on the data management, collection, analysis, use and interpretation (Campostrini, 2003).

While it is considered most ideal to collect data across short timeframes (e.g. a day, a week or a month) in order to simulate as closely as possible a continuous data collection, this is not always practical or feasible (Campostrini, 2003; McQueen, 2003). However, if questions and methodology remain stable over time so that changes or trends that occur can be attributed to true population changes and not questionnaire changes, and data are collected at regular intervals (McQueen, 2003); the information provided is extremely powerful.

Developing a systematic approach to surveillance addresses many needs. These include: an estimation of the size of the problem, the geographic distribution, detection of an epidemic or definition of a problem, stimulation of research and research hypotheses, monitoring changes in disease patterns and providing assistance to planning. Population-based

information related to economic, social, cultural and physical factors which are relevant to health can be provided. These factors can then be associated with the effects of public health and health promotion interventions and targeted campaigns. Data on health risk factors can also be obtained and support afforded to health related legislative programs and disease prevention actions, and regular surveillance can also evaluate the long term effects of health promotion campaigns. Also, future trends, the use of health resources and the emergence of any new health issues can be recognised (International Union for Health Promotion and Education World Alliance for Risk Factor Surveillance Global Working Group [IUHPE WARFS GWG], 2011).

Thus, while self-report underestimates osteoporosis prevalences, use of this method of data collection can improve the understanding and knowledge of the disease, in addition to assisting the identification of high risk groups (Werner, 2003). Self-report has been used in population surveys in South Australia (SA), Australia for approximately 20 years, with osteoporosis data being collected since 1995. Questions have been asked in the same way, using the same methodology annually, and while this timeframe is considered to be infrequent in terms of the surveillance timeframe spectrum (IUHPE WARFS GWG, 2011), aggregated data from these surveys possess the characteristics of a more regular surveillance system. Aggregation of these data provides the ability to analyse data over time and enables an assessment of changes in prevalence over the period of time under examination. It also provides evidence for the development of policy and an investigation of the impact of these policies over time.

3. Methods

The data presented in this chapter are derived from two different sources. The first is a face-to-face survey and the second, a telephone survey and clinical assessment conducted as part of a longitudinal cohort study.

3.1 Health Omnibus Survey (HOS)

The self-reported prevalence of osteoporosis has been collected in SA since 1995, using the SA Health Omnibus Survey (SAHOS) which is conducted annually, with data collection between September and December each year (spring to summer in Australia). Key uses of the survey are:

- gaining information on knowledge, attitudes and behaviours;
- gaining information on perceptions towards, and acceptability of, services and programs or organisations;
- provision of prevalence or incidence data;
- explaining population perspectives, attitudes, values and behaviours associated with issues under investigation;
- allowing the segmentation of problems and related issues;
- identifying target groups for interventions and campaigns;
- monitoring changes in health problems and disease trends;
- gaining information on the acceptability and uptake of new initiatives and programs;
- obtaining information on the aetiology of specific health problems;
- obtaining data to test hypotheses; and
- evaluating interventions and programs.

Questions to be included in each survey are reviewed by a quality control committee, both before and after pilot testing, for appropriate wording and design. Approximately ten background demographic questions are included within the survey. SAHOS is a face-to-face survey, which is the original method and consequently the "gold standard" of interview techniques (Dillman, 1999; Dillman et al., 2009; Schonlau et al., 2002). Participation is voluntary. Interviewers read out the questions to participants and, if necessary, prompt cards are used to ensure that respondents remember all of the response categories. The questionnaire is designed to take approximately 30 to 40 minutes for respondents to complete. Prior to the main survey, a pilot study of 50 interviews is conducted to test questions, validate the survey instrument and assess survey procedures.

3.1.1 Sample size

The survey sample is a clustered, multi-stage, systematic, self-weighting, area sample. Each of these key sampling concepts is described in more detail below. Each survey usually samples 5,200 households. The SAHOS has been in operation since 1991 and since that time the observed response rate has generally ranged between approximately 60-70%, usually resulting in a minimum of 3,000 interviews being completed each year. This large sample size facilitates a high level of confidence that the results and trends obtained in response to the survey questions can be extrapolated to the South Australian population as a whole.

3.1.2 Clustered sample

Seventy-five percent of the sample is selected from from the metropolitan area of the capital, Adelaide, with the remaining sample being drawn from those country areas with a population of 1,000 or more (based on Australian Bureau of Statistics (ABS) Census information which is collected every five years in Australia). Country towns with smaller populations are not included within the sample frame because of the additional cost of interviewing people living in these remote areas. Within the selected metropolitan and country areas, the ABS Collection Districts (CDs) are the basis of the sample frame. A CD is a geographical area comprising approximately 200 dwellings. By using a cluster sampling technique, some, but not all, of these CDs are included in the sample. To achieve a sample of 5,200 households, 10 households are selected from each of 520 CDs.

Stage 1 - Selection of CDs

Based on ABS population estimates, 400 CDs are selected in metropolitan Adelaide, and 120 CDs from the selected country areas. All cities/towns in country SA with a population size of 10,000 or more are selected automatically with the balance of the country sample chosen from centres with a population of 1,000 or more. A randomly selected starting point and a fixed skip interval are used to determine which CDs are chosen from the sample frame. The skip interval is calculated as the number of households in metropolitan Adelaide (or country SA) divided by the number of CDs required for the metropolitan (or country) sector.

The process of selection is as follows. Firstly, all CDs in the sample frame are listed in numerical code order, along with the number of dwellings in that individual CD and the "cumulative number of dwellings" for that CD. The cumulative number of dwellings is defined as the total number of dwellings for a particular CD and all previously listed CDs. A random number between one and the skip number is chosen as the starting point for selections and the skip interval is then used to determine which CDs are selected. If, for

example, the starting point is 80 and the skip interval is 100, then the CDs which contain the 80th, 180th and 280th cumulative dwelling will be the first three CDs to be selected. Thus, once the skip interval has been determined, selection of an individual CD is dependent on the number of dwellings within that CD. In some cases, larger CDs may, in theory, be selected more than once.

Stage 2 - Selection of households within CDs

The selection process of households is similar to the selection of CDs. Ten households per selected CD are chosen using a fixed skip interval from a random starting point.

Stage 3 - Selection of individuals within households

Within households, the person who was last to have a birthday (aged 15 years or over) is selected to participate in the survey. The sample is a non-replacement sample, thus, if the selected person is not available, interviews are not conducted with any other household members. Generally up to six visits are made to each household to interview the selected participant, before the selected individual is classified as a non-contact, however in some cases more visits may be conducted. Selections that occur in hotels, motels, hospitals, nursing homes and other institutions are excluded from the survey.

3.1.3 Systematic sample

The randomly selected starting points and the skip intervals between selected CDs and selected households within CDs produce a systematic even spread of households across the population.

3.1.4 Self weighting sample

The self-weighting sampling procedure of HOS ensures that every household within each of the two strata (metropolitan Adelaide and the major country towns) have the same probability of being selected even though different probabilities of selection exist at each stage of the sampling process.

The probability of selecting a household equals the probability of selecting a CD (i.e. the cumulative number of dwellings in the CD divided by the skip interval) multiplied by the probability of selecting a household, given that the CD was selected (i.e. the number of households required in each CD divided by the cumulative number of households in the CD).

3.1.5 Approach letter

In line with other epidemiologically-based surveillance systems, a letter introducing SAHOS is sent to each selected household including a brochure outlining how the information is used. It has been shown that sending a letter informing a person of a survey can increase response rates (Frey, 1989; Robertson et al., 2000). If respondents have any questions about the survey, they are able to call a free call telephone number listed in the approach letter.

3.1.6 Validation

Ten percent of all respondents are re-contacted and re-interviewed using selected questions to ensure the validity of the original responses. Data entry is fully verified using a double entry technique to ensure the accuracy of the final data.

3.1.7 Weighting

All SAHOS data are weighted by age, sex, area of residence and the inverse of the probability of selection in the household to the most recent ABS Census or Estimated Residential Population data for SA.

3.1.8 Ethics approval

Ethics approval for the methodology of the survey is provided by the Human Research Ethics Committee of the University of Adelaide and ethics approval for questions may be provided through the individual users' institutions, or if users do not have access to a committee, by the University of Adelaide.

3.1.9 Questions related to osteoporosis prevalence

The methodology of the SAHOS has remained consistent over time and questions relating to self-reported, doctor diagnosed osteoporosis have been included since 1995, which enables examination of prevalence changes over time.

Questions within the SAHOS include demographic characteristics:

- Sex;
- Age;
- Country of birth;
- Marital status;
- Income (gross annual household income before tax in Australian dollars);
- Work status;
- Area of residence; and
- Year of survey.

The question used to determine osteoporosis prevalence is "Have you ever been told by a doctor that you have osteoporosis? "

3.2 North West Adelaide Health Study

The North West Adelaide Health Study (NWAHS) is a longitudinal cohort study of over 4,000 participants located in the northwest suburbs of Adelaide, SA, Australia.

The study focuses on priority health conditions and risk factors that have been identified due to the significant burden that is placed on the community in terms of social, health, quality of life and economic factors. By identifying and describing specific population groups at risk of chronic conditions, the effectiveness of strategies for the prevention, early detection, and management of chronic conditions may be maximised (Grant et al., 2006; Grant et al., 2009).

Participants were recruited to Stage 1 of the study between 2000 and 2003, and undertook a second assessment between 2004 and 2006. The initial objective of the study was to establish both baseline self-reported and biomedically measured information on chronic diseases and risk factors, in terms of those who may be at risk of these conditions, those who already had these conditions but had not been diagnosed, and those who had previously been diagnosed with the conditions. Identifying those categories of disease along a chronic disease continuum provides a view of disease burden and presents opportunities for effective interventions, improved health service use and development of health policy (Grant et al., 2006; Grant et al., 2009). When specifically considering osteoporosis, the chronic disease continuum can be described as presented in Figure 1.

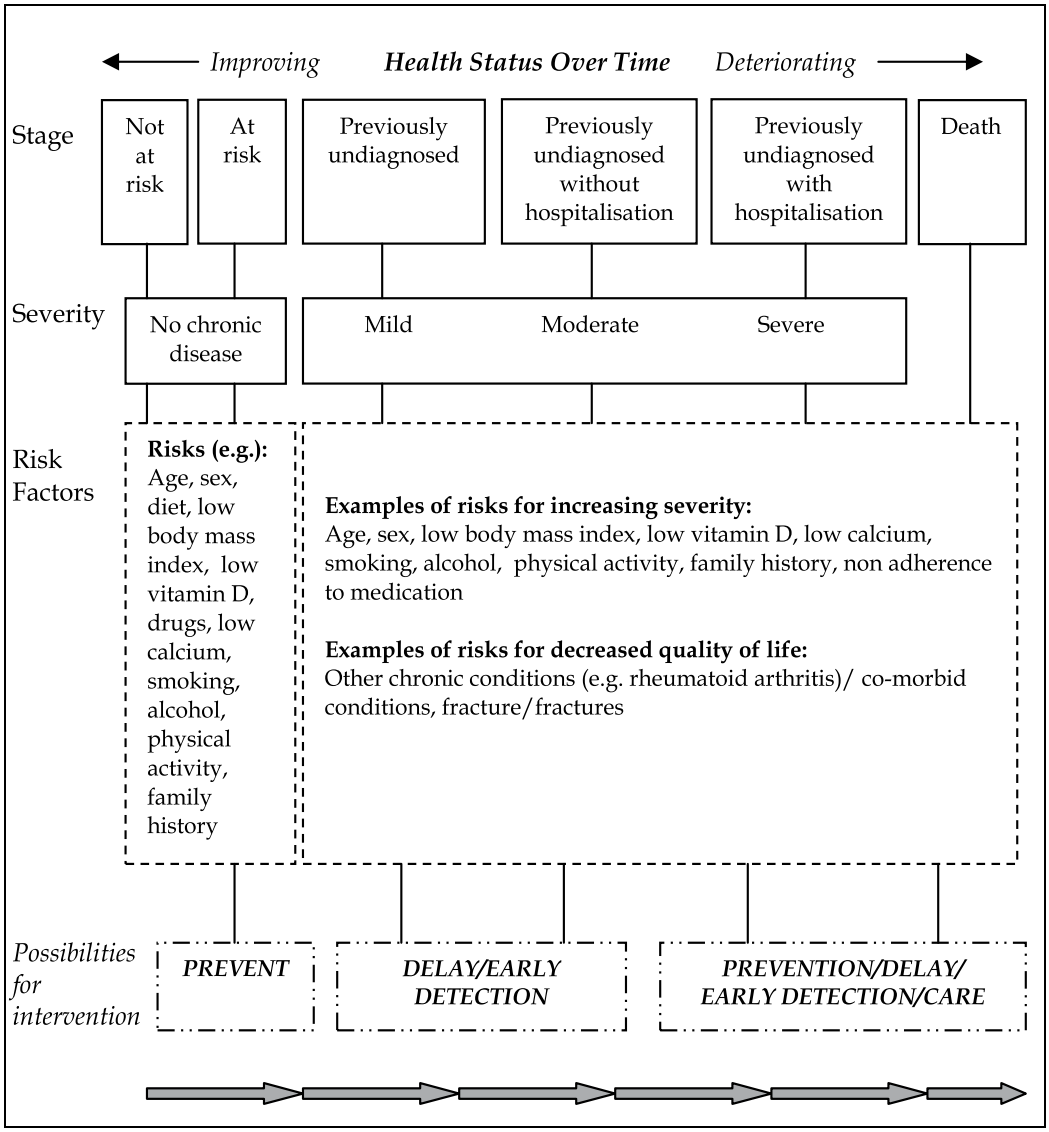

Fig. 1. Chronic Disease Continuum for Osteoporosis

3.2.1 Stage one

All households in the northern and western areas of Adelaide, SA, with a telephone connected and a telephone number listed in the Electronic White Pages were eligible for selection in the NWAHS. Households were randomly selected and sent an approach letter and brochure informing them about the study. The person who was last to have their birthday within each household and aged 18 years and over was selected for interview. Interviews were conducted using Computer Assisted Telephone Interview (CATI) technology.

During the telephone interview respondents were asked a range of health-related and demographic questions, and were invited to attend an assessment clinic for a 45 minute

appointment at either of two local hospitals in Adelaide, one in the western suburbs and one in the northern suburbs. All study participants who agreed to attend the clinic were sent an information pack about the study, including a self-report questionnaire which examined other chronic conditions and health-related risk factors that were not included in the telephone interview.
During the clinic visit, the tests included: height, weight, waist and hip circumference, and blood pressure. Lung function was calculated and a fasting blood sample was taken to measure glucose, tryglycerides, total cholesterol, high density lipid (HDL), low density lipid (LDL), and glycated haemoglobin (HbA1c). The response rate for attending the clinic in Stage 1 was 49.4% with a final sample of n=4056.

3.2.2 Stage two

All participants that could be contacted, were invited to attend the clinic for Stage 2 using a telephone interview that also obtained demographic and health-related information. Of the original living cohort, over 90% provided some Stage 2 information, and 3,205 (over 81.0%) attended the clinic assessment between 2004 and 2006 for the second time. The minimum age of participants in Stage 2 was 20 years. In addition to the measurements taken at Stage 1 (which concentrated on the chronic conditions diabetes, chronic obstructive pulmonary disease and asthma), renal function and musculoskeletal conditions were also assessed. The musculoskeletal conditions included both arthritis and osteoporosis and a range of questions related to specific joint pain. Participants aged 50 years and over were offered a DXA scan to measure their bone density, and fat and lean body mass.
The longitudinal nature of the cohort study means that following Stage 2, valuable information was obtained relating to the number of people who had developed chronic conditions over the timeframe of the study and the factors that may have contributed to their risk of developing chronic disease. Stage 3 of the study has recently been completed with all respondents who could be contacted again being asked to attend the clinic for assessment and information relating to musculoskeletal conditions again included in the study. However the results in this chapter are limited to Stage 2 data only.

3.2.3 Weighting

Weighting was used to correct for the disproportionality of the original sample with respect to the population of interest. The data were weighted for age, sex, probability of selection in the household and area of residence. These weights reflect any unequal sample inclusion probabilities and compensate for differential non-response. The data were weighted using the ABS Census data so that the health estimates calculated would be representative of the adult populations of the north west area of Adelaide. Subsequently, each stage of the study is weighted with the initial sample weight as the foundation figure.

3.2.4 Ethics approval

Ethics approval for the each stage of the NWAHS has been granted by the Ethics of Human Research Committee of The Queen Elizabeth Hospital, Adelaide, SA.

3.2.5 Questions related to osteoporosis prevalence

Data collection methods for the NWAHS are a CATI, a self-complete questionnaire and a clinic assessment. Questions incorporated within the NWAHS include demographic characteristics:

- Sex;
- Age;
- Country of birth;
- Marital status;
- Income (gross annual household income before tax in Australian dollars);
- Work status; and
- Area of residence.

The question used to determine osteoporosis prevalence is "Have you ever been told by a doctor that you have osteoporosis?" This information is collected as part of the CATI in Stage 2. Other information collected as part of the CATI was the self-reported occurrence of fractures following a fall from a standing height or less in the past year and self-reported types of arthritis, including rheumatoid and osteoarthritis (that is, "Have you ever been told by a doctor that you have arthritis?"). Those who responded in the affirmative were then asked what type of arthritis they had.

Other variables that are collected as part of the NWAHS were: family history of osteoporosis (mother, father, sister, brother, grandparent, other), self-reported smoking (which is categorised as current, ex- or non-smoker) and alcohol intake. Regarding alcohol intake, participants were asked how often they drank alcohol, and if they drank, on a day when they drank alcohol, how many drinks they usually had. They were then classified according to their level of risk of harm from alcohol, as non-drinkers or no risk, low alcohol risk, and intermediate to very high alcohol risk (National Heart Foundation of Australia, 1989). Physical activity level was also determined, respondents were asked about the amount of walking, moderate and vigorous activity they had undertaken in the past two weeks. These questions were the same as those used in the Australian National Health Survey in 2001 and 2004 (ABS, 2003, 2006), and the responses were classified into four activity levels (sedentary, low, moderate and high). All of these variables were obtained from the self-completed questionnaire.

Height and weight were measured as part of the clinic assessment to calculate body mass index and DXA scans were provided to those aged 50 years and over who consented to the scan and respondents were classified as having osteoporosis (T score ≤ -2.5) or osteopenia (-1.0 < T score > -2.5) using the World Health Organization (WHO) definition of osteoporosis (WHO, 1994). Overall 75.7% of eligible participants undertook a DXA scan.

3.3 Data analysis

Analyses were conducted using SPSS Version 18 (IBM SPSS Statistics, New York, NY, USA) and STATA Version 11.2 (StataCorp, College Station, TX, USA).

4. Results

4.1 Prevalence of osteoporosis (SAHOS)

The self-reported prevalence of osteoporosis has been collected every year in SAHOS between 1995 and 2010 except in 1996 and 2000. Thus there are fourteen years of data available. The aggregated sample size was n=41,487. Overall, 49% of respondents were male and 51.0% female, with a mean age of 45.0 years (SD 18.85, range 15-102). The aggregated prevalence of self-reported osteoporosis among those aged 15 years and over, between 1995 and 2010, was 4.8% (95% CI 4.6-5.0).

The self-reported prevalence from SAHOS was then age and sex standardised to the 2006 Australian Census (ABS, 2007) to enable prevalence comparisons between years and the results are presented in Figure 2. Data points for 1996 and 2000 are not available as these years had missing data.

As the data were aggregated, autocorrelation may occur which violates the assumptions of linear analysis. A Durbin-Watson test was undertaken to determine if first order autocorrelation of the residuals of the annual prevalence estimates had occurred. The value was 1.73, close to 2 indicating that there was not excessive autocorrelation of the data (Chatfield, 2004; Yaffee & McGee, 2000).

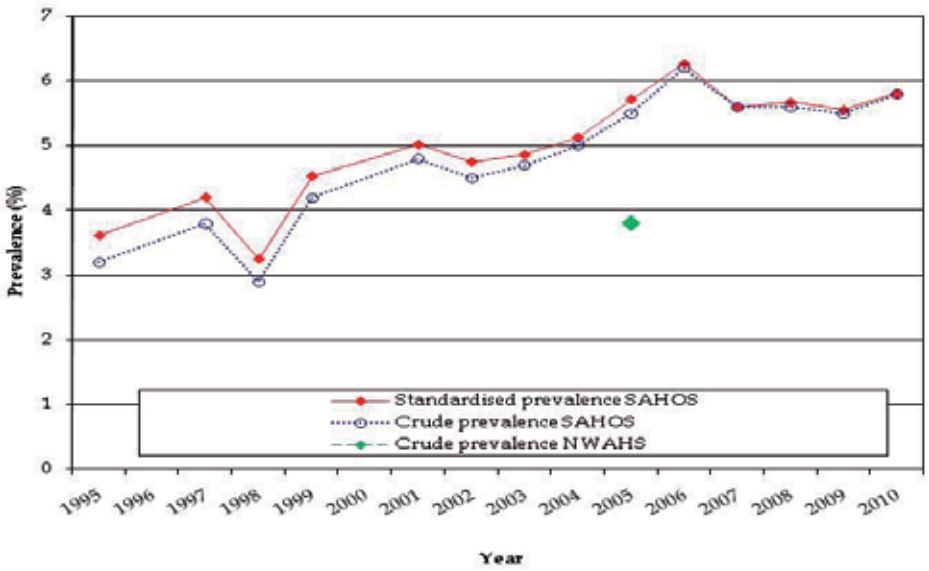

Fig. 2. Prevalence of self-reported osteoporosis from SAHOS and NWAHS

The data were examined to determine if a deviation from a linear trend existed. The regression coefficients were graphed and demonstrated an approximate straight line and a Box-Tidwell regression model was undertaken (Box & Tidwell, 1962), which also indicated that the nonlinear deviation was not significant ($p=0.09$). A chi-square test for trend was then conducted, which indicated that there had been a significant change in the self-reported osteoporosis prevalence over time ($p<0.001$).

4.2 Prevalence of osteoporosis (NWAHS)

Participants in Stage 2 of the NWAHS undertook one, two or all three of the data collection methods (CATI, self-complete questionnaire, clinic assessment) depending on their time constraints and desired level of participation. There were n=3500 respondents to the CATI questionnaire (49.1% male and 50.9% female; mean age 47.42, SD 17.57, range 20-93), n=3259 responded to the self-complete questionnaire (49.1% male and 50.9% female; mean age 47.59, SD 17.51, range 20-95) and n=3205 attended the clinic (49.1% male and 50.9% female; mean age 47.58, SD 17.52, range 20-95). The self-reported prevalence of osteoporosis among participants in the NWAHS aged 20 years and over during Stage 2 (2004 to 2006) was 3.8% (95% CI 3.2-4.5). The crude prevalence of osteoporosis obtained from the NWAHS is shown in Figure 2.

4.3 Logistic regression analyses

Logistic regression analysis of the aggregated SAHOS data set was then undertaken in order to determine the demographic characteristics most likely to be associated with self-reporting the presence of osteoporosis. Data for these analyses were restricted to respondents aged 20 years and over to enable comparisons with the NWAHS data. The variables included in the analysis were: age, sex, country of birth, income, education, marital status and work status. Area of residence was not included as SAHOS is a state wide sample and NWAHS is a metropolitan sample. Bivariate and then multivariate logistic regression analyses were conducted to identify the best sets of explanatory variables associated with osteoporosis, with variables that were significant at $p<0.25$ in the bivariate analysis included in the multivariate model, as these may still be candidates for model predictors - they can continue to be a good fit when other variables are included in the model (Hosmer & Lemeshow, 2000). Then the non-significant variables at $p\geq0.05$ were removed until all remaining variables were significant. Finally, all models were tested for "goodness of fit" using the Hosmer and Lemeshow goodness of fit test (Hosmer & Lemeshow, 2000).

Analysis of the demographic variables associated with self-reported osteoporosis for the SAHOS and NWAHS produced similar factors, with increasing age, sex (female) and work status (unemployed, retired and "other") significant for both datasets (Table 1). In the SAHOS, those who reported that they undertook home duties were also significantly more likely to report that they had osteoporosis. Those earning between $12,001 and $50,000, were also significantly more likely to self-report osteoporosis in the SAHOS, whereas this variable was not significant for those self-reporting osteoporosis in the NWAHS.

A model was then created for the SAHOS data only, to examine the impact of time. The variables associated with self-reporting osteoporosis were: increasing age, sex (female), work status (unemployed, retired and "other", home duties), income (up to $50,000 and not stated) and year, with more recent years associated with higher self-reported prevalence of osteoporosis (data not shown).

The self-reported prevalences collected as part of the SAHOS and the NWAHS were then compared to the prevalence of osteoporosis and osteopenia as defined in the NWAHS using DXA scans. DXA scans were only undertaken on those aged 50 years and over, thus the analysis is limited to this age group. The aggregated prevalence of self-reported osteoporosis among those aged 50 years and over in SAHOS was 10.7% (95% CI 10.3-11.2) compared to 8.8% (95% CI 7.5-10.3) for self-report in NWAHS and 18.7% (95% CI 16.6-20.9) for those classified with osteoporosis and osteopenia combined as defined by DXA scans (osteoporosis 3.6% (95% CI 2.6-4.9) and osteopenia 15.1% (95% CI 13.2-17.1)). It was considered appropriate to combine the categories of osteoporosis and osteopenia as both are indicators of abnormal bone density.

Bivariate and multivariate analyses were then undertaken for all participants aged 50 years and over. This second group of models included all of the demographic characteristics collected as part of both studies and examined the variables associated with self-report and clinically defined osteoporosis (Table 2). Increasing age and female sex were significant for all three models and again for SAHOS and self-reported osteoporosis from NWAHS, work status (unemployed and retired) was significant. Income was also significantly associated with self-reporting osteoporosis in SAHOS, while marital status (never married) was significantly associated with low bone density as defined by DXA scans.

	SAHOS self-report		NWAHS self-report	
Group One	OR	p-value	OR	p-value
Sex				
Male	1.00		1.00	
Female	3.76	**<0.001**	5.55	**<0.001**
Age	1.06	**<0.001**	1.06	**<0.001**
Work status				
Full time	1.00		1.00	
Part time	1.13	0.432	1.48	0.430
Home duties	1.47	**0.009**	1.16	0.866
Unemployed	2.05	**0.024**	3.25	**0.015**
Retired	1.44	**0.015**	2.95	**0.037**
Student	1.40	0.436	-	-
Other	5.65	**<0.001**	4.43	**0.020**
Not stated	-	-	4.84	0.108
Income				
$80,001 and more	1.00			
$60,001 - $80,000	1.41	0.108		
$50,001 - $60,000	1.23	0.362		
$40,001 - $50,000	1.80	**0.004**		
$30,001 - $40,000	1.61	**0.022**		
$20,001 - $30,000	1.68	**0.006**		
$12,001 - $20,000	1.72	**0.004**		
Up to $12,000	1.33	0.129		
Not stated	1.31	0.146		

Table 1. Logistic regression analysis of self-report osteoporosis, SAHOS and NWAHS, age 20 years and over

A model was also constructed for those aged 50 years and over using SAHOS data only, which examined the demographic characteristics associated with self-reported osteoporosis and included year within the model. Increasing age, female sex, increasing years, work status (home duties, retired, student, other) and income (up to $50,000) were all significant and associated with self-reporting osteoporosis (data not shown).

A third group of models was then created using NWAHS data and examining other factors associated with osteoporosis. Variables examined at a bivariate level were alcohol risk, smoking, family history, body mass index, physical activity, fracture as a result of a fall from

a standing height or less over the last year and self-reported rheumatoid arthritis. The results of the multivariate analysis are in Table 3.

	SAHOS self-report		NWAHS self-report		NWAHS DXA	
Group two	OR	p-value	OR	p-value	OR	p-value
Sex						
Male	1.00		1.00		1.00	
Female	4.44	**<0.001**	5.50	**<0.001**	1.94	**<0.001**
Age	1.04	**<0.001**	1.03	**0.020**	1.09	**<0.001**
Work status						
Full time	1.00		1.00			
Part time	1.16	0.46	1.12	0.823		
Home duties	1.72	**0.003**	0.93	0.934		
Unemployed	2.05	**0.035**	3.04	**0.015**		
Retired	1.75	**0.002**	2.67	**0.034**		
Student	3.25	**0.018**	-	-		
Other	6.08	**<0.001**	2.57	0.180		
Not stated	-	-	4.69	0.081		
Income						
$80,001 and more	1.00					
$60,001 - $80,000	1.31	0.295				
$50,001 - $60,000	1.14	0.632				
$40,001 - $50,000	1.87	**0.012**				
$30,001 - $40,000	1.50	0.106				
$20,001 - $30,000	1.55	0.051				
$12,001 - $20,000	1.60	**0.038**				
Up to $12,000	1.28	0.272				
Not stated	1.18	0.465				
Martial status						
Married/de facto					1.00	
Separated/divorced					0.80	0.345
Widowed					1.28	0.253
Never married					1.90	**0.043**
Not stated					0.86	0.903

Table 2. Logistic regression analysis of osteoporosis prevalence, SAHOS and NWAHS self-report and NWAHS DXA, age 50 years and over

For both self-report and DXA, non-smokers were more likely to have osteoporosis. Those with a low to high risk of harm from alcohol and with a first degree relative with osteoporosis were more likely to self-report that they had osteoporosis whereas those undertaking lower levels of activity were more likely to have low bone density. Those with a higher body mass index were less likely to have a low bone density (Table 3).

	NWAHS self-report		NWAHS DXA	
Group three	OR	p-value	OR	p-value
BMI			0.82	**<0.001**
Alcohol risk				
Non drinker/no risk	1.00			
Low to high risk	1.54	**0.025**		
Not stated	1.34	0.593		
Smoking				
Current smoker	1.00		1.00	
Ex smoker	1.21	0.629	1.13	0.637
Non smoker	2.37	**0.026**	1.80	**0.020**
Family history of osteoporosis				
No	1.00		1.00	
First degree relative	3.66	**<0.001**	1.43	0.095
Don't know	2.73	**<0.001**	1.45	**0.037**
Not stated	1.13	0.851	2.20	0.124
Physical activity				
High exercise			1.00	
Moderate exercise			1.71	0.144
Low exercise			2.07	**0.042**
Sedentary			2.15	**0.034**
Not stated			2.40	**0.027**

Table 3. Logistic regression analysis of other factors associated with osteoporosis, NWAHS self-report and DXA, age 50 years and over

Finally a fourth set of models combined both demographic and other relevant factors associated with osteoporosis, for the data obtained from the NWAHS. Increasing age and female sex remained significant for both models, while those with a higher body mass index were less likely to have a lower bone density and those with self-reported osteoporosis were more likely to have family members with the condition. The work status categories, unemployed and retired, also remained significant for self-reported osteoporosis (Table 4).

	NWAHS self-report		NWAHS DXA	
Group four	OR	p-value	OR	p-value
Sex				
Male	1.00		1.00	
Female	4.71	**<0.001**	2.13	**<0.001**
Age	1.03	**0.013**	1.10	**<0.001**
BMI			0.82	**<0.001**
Work status				
Full time	1.00			
Part time	1.05	0.922		
Home duties	0.78	0.775		
Unemployed	2.85	**0.023**		
Retired	2.55	**0.044**		
Student	-	-		
Other	2.19	0.275		
Not stated	6.22	**0.023**		
Family history				
No	1.00			
First degree relative	3.24	**<0.001**		
Don't know	2.17	**0.001**		
Not stated	0.97	0.962		

Table 4. Overall models demographic and other factors associated with osteoporosis, NWAHS, age 50 years and over

5. Discussion

The results of this analysis indicate that while determining the population prevalence of osteoporosis remains a difficult issue, there is a role for self-report to play in the monitoring of osteoporosis prevalence over time. At this time, DXA scans are not available to the general population as a screening tool (Davis et al., 2011) and other means of assessing osteoporosis in the population are required. However, it is likely, as this study has shown, that self-reported prevalence will differ from that obtained from bone density assessment. In this study, the prevalence of osteoporosis as measured by DXA among those 50 years and over was lower than the self-reported prevalence but when combined with osteopenia was higher. Sample differences and self-selection to undertake a DXA scan are likely to have contributed to this.

Data from the SAHOS indicate that there has been a significant increase in the self-reported prevalence of osteoporosis over time. It is however difficult to assess whether this is a true increase. Other factors such as a greater awareness of the condition due to marketing campaigns in Australia, particularly in relation to over the counter supplements such as

vitamin D and calcium, may have played a role in increasing the awareness of the condition. Nevertheless, it can also be argued that this improved awareness, even if it impacts estimates of true prevalence, may still assist in the prevention or management of the condition. Nayak et al. (2010) demonstrated that belief in being susceptible to osteoporosis among older adults, those most at risk of osteoporosis, is low, and the older the respondents were, the less likely they were to believe that osteoporosis is a severe condition. Thus any information or advertising may assist patient education. It is also of interest that the results of DXA scans are not immune to errors in self-report with Cadarette et al. (2007) demonstrating that while there was minimal error in self-reporting that a DXA scan had been undertaken, the self-reporting of results was poor, again providing an underestimate of osteoporosis prevalence. The understanding of these results could however be improved by providing them in writing (Brask-Lindemann et al., 2011). This again highlights issues of patient knowledge and understanding of the condition.

Consequently, in recent years, there has been an increased focus on functional health literacy of the population, which is considered to be the ability of people to read, analyse and take action with regard to both oral and written information obtained in the health care setting (Nielsen-Bohlman et al. 2004). It has been acknowledged that those with low or limited functional health literacy are more likely to have adverse health outcomes, not undertake preventive health behaviours, have premature mortality and higher health-care costs. In addition, people with lower functional health literacy are less likely to undertake active management of their condition (Berkman et al., 2004; De Walt et al., 2005). Unpublished analysis of other data obtained using the SAHOS has indicated that 70% of those with doctor-diagnosed osteoporosis had a low health literacy, further supporting the view that despite the method used to assess osteoporosis prevalence, inaccuracies in the reporting of the condition may occur, which has implications for management of the condition.

While understanding of the condition and reporting of prevalence is variable, it is of note that there remained a general consistency in the variables that were associated with osteoporosis prevalence, and these are supported by previous work. Genetic factors have been shown to contribute to osteoporosis (Harvey & Cooper, 2003; Marini & Brandi, 2010; Recker & Deng, 2002), thus family history of osteoporosis is an important factor. Sex and age are also significant covariates (Cawthon, 2011; Keen, 2007; Werner, 2003) and these variables are strongly evident during multivariate modelling. Varenna et al. (1999) determined that higher levels of education were associated with a lower risk of osteoporosis and lower income levels and unemployment have been associated with a greater risk of hip fracture (Farahmand et al., 2000). Low body mass index, previous low trauma fracture, rheumatoid arthritis, physical activity, smoking and excessive alcohol consumption have all been identified as risk factors for osteoporosis (Keen, 2007). Despite the fact that many of these variables were self-report, the associations with osteoporosis all occurred in the expected manner, except for smoking, where non-smokers were more likely to have a lower bone density and to self-report osteoporosis. Smokers are more likely to be from lower socioeconomic groups (Scollo & Winstanley, 2008) which are also groups with a lower level of health literacy (Barber et al., 2009). Thus this group may be less likely to undergo a DXA scan and self-report osteoporosis, as they have a poorer understanding of the condition.

The consequences of osteoporosis in terms of fracture also need to be considered. The overall lack of awareness of osteoporosis within the population also extends to a lack of

understanding of the risk of a prior fracture in relation to the occurrence of subsequent fractures (Center et al., 2007). Targeted self-management courses for osteoporosis have demonstrated an improved understanding of osteoporosis and related behaviours in the short term (Francis et al., 2009; Laslett et al., 2011) and in Australia, approximately 40% of those with osteoporosis are more likely to use complementary and alternative medicines, which includes vitamin D and calcium (Armstrong el al., 2011). But despite public campaigns promoting better nutrition and increasing the awareness of osteoporosis, Pasco et al. (2000) demonstrated that women did not achieve the required calcium intake and Czernichow et al. (2010) have shown that the vitamin D intake among postmenopausal women with osteoporosis in France is significantly lower than recommended doses.

As highlighted in this study, similar factors were associated with osteoporosis prevalence, notwithstanding the method of data collection. Thus, surveillance can play a role in the ability to target information, identify at-risk groups and evaluate the impact of health promotion programs. It is however evident that there is a continued need to further explore means of adequately ascertaining the prevalence of osteoporosis and to improve the understanding of the condition in the population.

6. Conclusion

While prevalence estimates of osteoporosis vary within the population according to data collection method, generally there are consistent covariates associated with osteoporosis, which are important for the targeting of health promotion campaigns. In the absence of clinical testing, the monitoring of the prevalence of osteoporosis using self-report has a role to play in the prevention and management of the condition.

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5

Prevalence of Back Pain in Postmenopausal Osteoporosis and Associations with Multiple Spinal Factors

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1. Introduction

Back pain is considered to be most prevalent musculoskeletal pain, particularly in elderly populations (Woo et al., 2009). The existing literature suggests a prevalence of chronic back pain among the elderly ranging from 7% to 58% (Edmond & Felson, 2000; Jacobs et al., 2006; Lavsky-Shulan et al., 1985; March et al., 1998), with differences attributable to a lack of concordance in terms of age stratification, definition, and methodology, but with consistently much higher rates in women than men (Jabobs et al., 2006; Woo et al., 2009). The reason why back pain is common among elderly women may be related to osteoporosis. As lower bone mineral density (BMD) and the rapid decline in BMD following menopause in women result in a greater prevalence of osteoporosis and vertebral fractures compared to men, osteoporosis is likely to represent a major cause of back pain among elderly women. However, although osteoporosis may be an underlying cause of back pain, especially in postmenopausal elderly women, the prevalence of back pain in this group has not been fully investigated.

Although back pain in osteoporosis is often attributed to vertebral fractures (Nevitt et al., 1998; Ulivieri, 2007), the intensity of pain is not always influenced by fracture status (Hübscher et al., 2010). Liu-Ambrose et al. demonstrated that osteoporotic women may experience back pain without a concomitant history of vertebral compression fractures (Liu-Ambrose et al., 2002). The cause of back pain in osteoporosis thus seems likely to be related to multiple factors.

Spinal alignment and mobility are important factors for spinal function and may be related to back pain. Loss of lumbar lordosis correlates well with the incidence of chronic low back pain in adults (Djurasovic & Glassman, 2007; Glassman et al., 2005). Patients with a less mobile spine may show more severe symptoms. In addition, we have previously demonstrated that back extensor strength is significantly associated with spinal mobility (Miyakoshi et al., 2005). However, to the best of our knowledge, simultaneous assessment of back pain and multiple spinal factors such as vertebral fractures, spinal alignment and mobility, as well as back extensor strength, has not yet been investigated in patients with osteoporosis.

The objectives of this study were thus: 1) to determine the prevalence of back pain in patients with postmenopausal osteoporosis who visited their practitioner; and 2) to evaluate

associations of back pain and vertebral fractures, spinal alignment, mobility, and back extensor strength in these patients.

2. Materials and methods

2.1 Patients

A total of 174 consecutive women with postmenopausal osteoporosis aged 50 years and older who visited their practitioner (orthopedic clinic) were enrolled in the present study. All these patients were the same patients who enrolled in our previous study assessing back extensor strength and quality of life (QOL) (Miyakoshi et al., 2007). Osteoporosis was diagnosed according to the criteria proposed by the Japanese Society for Bone and Mineral Research (JSBMR) (Orimo et al., 2001). Briefly, patients with BMD less than 70% of the young adult mean BMD or with fragility fracture were diagnosed as having osteoporosis. All participants were asked whether they had clinically relevant back pain, and BMD, number of vertebral fractures, angle of kyphosis, range of motion (ROM) of the thoracic and lumbar spine, and back extensor strength were evaluated. These variables were compared between subjects with back pain (BP group) and those without back pain (non-BP group). In the BP group, associations between intensity of back pain and other measured variables were further evaluated.

Exclusion criteria were as follows: 1) women with a history of metabolic bone disease, malignancy, or recent antiosteoporotic treatment (with exception of calcium); 2) patients with hip fracture; 3) patients who could not lie in a prone position; 4) chronic use of glucocorticoids; 5) a concomitant illness that would substantially influence the daily living (e.g., chronic pulmonary disease, asthma, angina, chronic congestive heart failure, stroke, blindness, etc.); 6) other diseases that might cause back pain (e.g., scoliosis, lumbar spondylolisthesis, lumbar disc disease, etc.); and 7) patients with documented vertebral fracture within the last 6 months. Patients enrolled in the present study thus showed chronic back pain that was not attributable to a fresh vertebral fracture.

2.2 Definition of clinically relevant back pain

Back pain was considered clinically relevant if the participant answered that pain had been moderately to severely bothersome, or if the participant needed any medical treatment (Miyakoshi et al., 2010; Nevitt et al., 1998). In this study, the definition of back was not limited to the narrow sense of the upper and middle back, and low back was also included, as patients with osteoporosis often complain of pain affecting both definitions and differentiating between these seems difficult (Satoh et al., 1988).

2.3 Evaluation of back pain intensity

Intensity of back pain was evaluated using the pain domain score of the Japanese Osteoporosis QOL Questionnaire (JOQOL) (Table 1) (Kumamoto et al., 2010; Takahashi et al., 2000); as all questions for this score are limited to back pain, all domain scores show significant correlations on test and retest (Kendall's τ = 0.691-0.818) (Kumamoto et al., 2010) and the score can be used as a continuous variable to evaluate correlations with other measured variables. The pain domain score of JOQOL contains 5 questions. Scores for each item range from 0 to 4, for a full score of 20. Pain intensity indicated in this study was calculated as 20 – estimated pain domain score of JOQOL. The pain intensity evaluated in this study thus ranged from 0 (no pain) to 20 (worst pain).

Question	Score (points)
How often have you had back or low back pain in the last week?	
Never	4
1. 1 day per week or less	3
2. 2-3 days per week	2
3. 4-6 days per week	1
4. Every day	0
If you have had back pain or low back pain, for how long did you have it in the daytime?	
1. No pain	4
2. 1-2 hours	3
3. 3-5 hours	2
4. 6-10 hours	1
5. All day	0
While you kept still, how severe was your back or low back pain?	
1. No pain	4
2. Mild	3
3. Moderate	2
4. Severe	1
5. Unbearable	0
When you moved, how severe was your back or low back pain?	
1. No pain	4
2. Mild	3
3. Moderate	2
4. Severe	1
5. Unbearable	0
Has the back or low back pain disturbed your sleep in the last week?	
1. Never	4
2. Once	3
3. Twice	2
4. Every other night	1
5. Almost every night	0
Total	20

*Reference from Kumamoto et al., 2010.

Table 1. Pain domain questions of the Japanese Osteoporosis Quality of Life Questionnaire (JOQOL)*

2.4 Evaluation of vertebral fractures

X-rays of the thoracic and lumbar spine in lateral views with the patient in a neutral/lateral decubitus position were taken with a film-tube distance of 1 m. Thoracic films were centered

on T8, while lumbar films were centered on L3 (Miyakoshi et al., 2003b). Anterior, central, and posterior heights of each vertebral body from T4 to L5 were measured using calipers (Miyakoshi et al., 2003b). Coefficient of variation for this measurement was 2-3% (Orimo et al., 1994). Vertebral fracture was considered present if at least one of the three height measurements (anterior, middle, or posterior) of one vertebral body had decreased by more than 20% compared with the height of the nearest uncompressed vertebral body (Orimo et al., 1994).

2.5 Measurement of spinal kyphosis angles and ROMs

Angles of kyphosis and ROM of the thoracic (T1-T12) and lumbar (L1-L5) spine were measured using a device for computerized measurement of surface curvature (SpinalMouse®; Idiag, Volkerswill, Switzerland) in an upright position and at maximum flexion and extension (Kasukawa et al., 2010; Miyakoshi et al., 2005). Details regarding this device have been provided elsewhere (Post & Leferink, 2004). The device consists of a mobile unit of 2 rolling wheels interfacing with a base station through telemetry. By sliding the mobile unit along the spinal curvature, sagittal spinal alignment is calculated and displayed on the computer monitor. Repeating this process with the patient in flexion and extension of the spine allows measurement of ROM (Post & Leferink, 2004). SpinalMouse® delivers consistently reliable values for standing curvatures and ROM (Mannion et al., 2004; Post & Leferink, 2004). Post and Leferink (Post & Leferink, 2004) reported that interrater intraclass correlation coefficients (ICCs) for curvature measurement with SpinalMouse® were greater than 0.92. Mannion et al. (Mannion et al., 2004) reported that intrarater ICCs ranged from 0.82 to 0.83, while interrater ICCs ranged from 0.81 to 0.86. In addition, our previous studies have shown that thoracic and lumbar angles of kyphosis and spinal ROM measured using the SpinalMouse® correlate strongly with those measured on spinal radiography ($r=0.804$, $r=0.863$, and $r=0.783$, respectively; $p<0.0001$) (Miyakoshi et al., 2004).

2.6 Measurement of BMD

BMD was measured by dual-energy X-ray absorptiometry (QDR-4500; Hologic, Bedford, MA). Measurements were obtained from anteroposterior projections of the second to fourth lumbar vertebrae, the femoral neck, and the whole body. The coefficient of variation for these variables in 5 corresponding measurements from 5 normal volunteers was less than 1.5% (Miyakoshi et al., 2007).

2.7 Measurement of back extensor strength

Isometric back extensor strength in prone position was measured using a strain-gauge dynamometer (Digital Force Gauge DPU-1000N; IMADA, Toyohashi, Japan) as previously described (Hongo et al., 2007; Limburg et al., 1991; Miyakoshi et al., 2005). Subjects were allowed one warm-up trial, followed by three successive maximal effort trials separated by 60-s rest periods (Hongo et al., 2007). Maximal force among the three trials was selected and documented. Coefficient of variation for this measurement was 2.3% (Limburg et al., 1991).

2.8 Data analysis

All data are presented as mean and standard deviation (SD). Statistical analysis was performed using StatView version 5.0 software (Abacus Concepts, Berkeley, CA). Statistical

differences between groups were compared using an unpaired t-test. Logistic regression analysis was used for analyzing significant risk factors for back pain. Correlations between pain intensity and other measured variables were analyzed using Pearson's correlation coefficient and simple regression analysis. Further analyses using multiple regression were conducted to determine which variables best correlated with back pain. Values of $P<0.05$ were considered statistically significant.

3. Results

In this study, among 174 patients with postmenopausal osteoporosis, 159 patients (91.4%) complained of back pain. Mean values for age and measured variables in the BP and non-BP groups are listed in Table 2. No significant differences were apparent between BP and non-BP groups with regard to age, BMDs, number of vertebral fractures, angles of thoracic and lumbar kyphosis, and thoracic and lumbar ROMs. However, back extensor strength was significantly lower in the BP group than in the non-BP group. Similarly, when univariate logistic regression analysis was performed with the presence of back pain as a dependent variable and the other estimated variables as independent variables, only back extensor strength was identified as an index significantly associated with the presence of back pain (Table 3).

In patients with back pain, correlations between pain intensity and measured variables were evaluated. Pain intensity showed a significant positive correlation with the number of vertebral fractures, and negative correlations with lumbar spinal ROM and back extensor strength (Table 4). However, no significant correlations were observed between pain intensity and age, BMDs of all measured sites, angles of thoracic and lumbar kyphosis, or thoracic spinal ROM. Based on these results, number of vertebral fractures, lumbar spinal ROM, and back extensor strength were selected as independent variables for multiple regression modeling of pain intensity. Multiple regression analysis for pain intensity revealed lumbar spinal ROM and back extensor strength as significantly associated with pain intensity (Table 5).

	BP (n=159)	Non-BP (n=15)	*P*
Age (years)	67.8±6.5	65.5±7.0	0.1819
Lumbar spine BMD (g/cm^2)	0.696±0.111	0.687±0.103	0.7757
Femoral neck BMD (g/cm^2)	0.550±0.087	0.542±0.070	0.7105
Whole-body BMD (g/cm^2)	0.818±0.075	0.812±0.047	0.7556
No. of vertebral fractures	1.2±1.7	0.3±0.5	0.0637
Thoracic kyphosis angle (degrees)	44.2±14.1	42.9±12.3	0.7326
Lumbar kyphosis angle (degrees)	-15.5±18.2	-23.7±16.8	0.0977
Thoracic spinal ROM (degrees)	17.5±12.8	19.7±19.7	0.5499
Lumbar spinal ROM (degrees)	51.3±17.6	57.3±14.6	0.2025
Back extensor strength (kg)	12.9±6.3	17.3±6.6	0.0130

Values represent mean ± SD. BP, patients with back pain; non-BP, patients without back pain; BMD, bone mineral density; ROM, range of motion.

Table 2. Comparison of estimated variables between osteoporotic patients with and without back pain.

	OR	95% CI	*P*
Age (years)	1.052	0.976-1.134	0.1845
Lumbar spine BMD (g/cm^2)	2.044	0.015-269.925	0.7741
Femoral neck BMD (g/cm^2)	3.289	0.006-1695.221	0.7086
Whole body BMD (g/cm^2)	3.275	0.002-5461.831	0.7540
No. of vertebral fractures	2.107	0.949-4.680	0.0670
Thoracic kyphosis angle (degrees)	1.007	0.969-1.046	0.7308
Lumbar kyphosis angle (degrees)	1.033	0.994-1.074	0.0958
Thoracic spinal ROM (degrees)	0.988	0.951-1.027	0.5475
Lumbar spinal ROM (degrees)	0.980	0.951-1.011	0.2033
Back extensor strength (kg)	0.906	0.835-0.982	0.0166

OR, odds ratio; CI, confidence interval; BMD, bone mineral density; ROM, range of motion.

Table 3. Univariate logistic regression analysis for back pain in patients with osteoporosis (n=174).

	Correlation coefficient *(r)*	*P*
Age (years)	0.118	0.1391
Lumbar spine BMD (g/cm^2)	-0.089	0.2643
Femoral neck BMD (g/cm^2)	-0.090	0.2583
Whole body BMD (g/cm^2)	-0.097	0.2258
No. of vertebral fractures	0.171	0.0312
Thoracic kyphosis angle (degree)	-0.043	0.5892
Lumbar kyphosis angle (degree)	0.139	0.0803
Thoracic spinal ROM (degree)	-0.109	0.1707
Lumbar spinal ROM (degree)	-0.264	0.0007
Back extensor strength (kg)	-0.268	0.0006

*Pain intensity ranged from 0 (no pain) to 20 (worst pain) was calculated from 20 minus estimated pain domain score of JOQOL. BMD, bone mineral density; ROM, range of motion.

Table 4. Correlations between pain intensity* and estimated variables in patients with osteoporosis and back pain (n=159).

	Coefficient *(r)*	*P*
Intercept	11.238	<0.0001
No. of vertebral fractures	0.132	0.4517
Lumbar spinal ROM (degrees)	-0.041	0.0179
Back extensor strength (kg)	-0.116	0.0142

*Pain intensity ranged from 0 (no pain) to 20 (worst pain), calculated as 20 minus the estimated pain domain score of JOQOL. ROM, range of motion.

Table 5. Multiple regression analysis for pain intensity* in patients with osteoporosis and back pain (n=159).

4. Discussion

4.1 Prevalence of back pain

Back pain is a major source of morbidity among patients with osteoporosis. Osteoporotic vertebral fractures usually cause acute, disabling, painful episodes at the fracture site. Such acute back pain subsides with fracture healing. However, after the fracture heals, the resulting increase in spinal kyphosis is likely to cause chronic back pain (Francis et al., 2008; Satoh et al., 1988). Increased spinal kyphosis is likely to induce abnormal stress on the supporting structures of the spinal column and may cause chronic back pain that usually develops while standing, walking, or doing other normal daily activities (Satoh et al., 1988). The back pain evaluated in the present study was considered to be chronic, because patients with documented vertebral fracture within the preceding 6 months were not included.

The prevalence of back pain, particularly chronic back pain, in patients with osteoporosis has not been fully investigated. Cockerill et al. (Cockerill et al., 2000) reported that the prevalence of back pain in the current and past year for women aged 50 years and over was significantly higher in women with single lumbar vertebral deformities (51.4% and 72.6%, respectively) than in women without vertebral deformity (39% and 60.6%, respectively) ($p<0.05$). Jacobs et al. (Jacobs et al., 2006) undertook a longitudinal study of 277 elderly subjects, finding that the prevalence of chronic back pain increased from 44% to 58% at ages 70 and 77 years, respectively, and this pain was associated with female sex at age 70 years and osteoporosis at age 77 years. More recently, Kuroda et al. (Kuroda et al., 2009) reported that back pain was observed in 28% of 818 Japanese postmenopausal women aged over 40 years (mean, 62.1 years) who visited their practitioner, and this back pain was associated with osteoporosis and vertebral fractures. In the present study, the prevalence of clinically relevant back pain was 91.4%. This percentage is higher than previously reported prevalences of back pain in osteoporosis (28-72.6%) (Cockerill et al., 2000; Jacobs et al., 2006; Kuroda et al., 2009), probably because all patients enrolled in the present study were visitors to an orthopedic clinic and might have had more musculoskeletal symptoms.

4.2 Factors associated with back pain and pain intensity

Previous studies have shown that vertebral fractures are associated with back pain and disability, with the strength of these associations increasing with the number and severity of fractures (Ettinger et al., 1992; Huang et al., 1996; Matthis et al., 1998). Increased spinal kyphosis caused by vertebral fractures is also known to induce back pain and disability in patients with osteoporosis (Miyakoshi et al., 2003a). In the present study, the number of vertebral fractures and angles of lumbar kyphosis tended to be higher in patients with back pain than in those without back pain, but no significant differences were identified ($p=0.0637$ and $p=0.0977$, respectively). However, in patients with back pain, the present study also showed a significant positive correlation between number of vertebral fractures and pain intensity ($r=0.171$, $p=0.0312$).

An important association between back pain and back extensor strength in patients with osteoporosis is indicated from the present study. Back extensor strength was significantly lower in patients with back pain compared to those without back pain, but other factors we evaluated showed no significant differences between groups. In addition, among patients with back pain, multiple regression analysis for pain intensity revealed back extensor strength and lumbar spinal ROM as significantly associated with pain intensity. Decreased back extensor strength may thus represent the most important factor contributing to back

pain and pain intensity in patients with osteoporosis. Subjects on acute back pain due to fresh vertebral fractures maybe could not perform the back extensor strength tests as good as non-acute pain subjects. However, because the back pain evaluated in the present study was considered to be chronic, all the patients could perform the strength tests without increasing the pain. Thus, we concluded that the weakness of back extensor is a very important factor for chronic back pain in patients with osteoporosis.

Back extensor strength reportedly shows a significant relation with spinal mobility (Miyakoshi et al., 2005), and decreased mobility of the spine is thought to lead to increased kyphosis and weakness of the paravertebral muscles, as well as the development of impaired physical function (Burger et al., 1997). Decreased back extensor strength may thus reduce mobility of the lumbar spine, and a less mobile lumbar spine may cause stiffness of the back muscles, resulting in back pain. As muscle strength is determined largely by muscle mass, particularly the cross-sectional area of muscle (Maughan, 2005), and because the muscle cross-sectional area of back extensor muscles (the erector spinae group) is larger at the lumbar spine level than at the thoracic spine level (Marras et al., 2001), total back extensor strength is largely influenced by lumbar extensor muscles rather than thoracic extensor muscles. The results of the present study are not inconsistent with this anatomical background. Weakness of the back extensor muscles, particularly the lumbar extensor muscles, is thought to be responsible for lumbar spinal mobility.

4.3 Other possible factors contributing to back pain in osteoporosis

The present study focused on back pain and multiple spinal factors in patients with osteoporosis. However, the etiology of back pain is more complex and more multifactorial than could be examined in this study. Prevalence of musculoskeletal pain is also known to be associated with various measures of socio-economic status, as well as comorbidities (Thomas et al., 1999; Woo et al., 2009). Severity of pain may also be influenced by psychological factors (Woo et al., 2009). In addition, elderly patients with osteoporosis sometimes show other painful spinal disorders such as spondylosis to varying extents (Miyakoshi et al., 2003b). Findings in the present study might also have been influenced, at least in part, by factors other than osteoporosis.

4.4 Study limitations

Limitations of the present study should be noted. First, the number of subjects in the present study was much smaller than in previous studies evaluating the prevalence of back pain (Cockerill et al., 2000; Jacobs et al., 2006; Kuroda et al., 2009). However, we would like to emphasize that this is the first study to simultaneously evaluate back pain and multiple spinal factors in patients with osteoporosis. Second, data could not be obtained from severely kyphotic patients with established osteoporosis who were too disabled to lie in a prone position because of increased back pain in this position. This was because the dynamometer for measuring back extensor strength in the present study needed the patient to lie in a prone position. Therefore, the results of the present study might be considered for patients with mild or moderate spinal deformity.

5. Conclusions

In conclusion, the prevalence of back pain among patients with postmenopausal osteoporosis ≥50 years old who visited their practitioner was 91.4%. Back extensor strength

was significantly lower in patients with back pain compared to those without back pain. Among subjects with back pain, intensity of back pain showed significant relationships with decreased back extensor strength and limited lumbar spinal mobility.

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Part 3

The Diagnosis and Assessment of Osteoporosis and Fractures Risk

6

The Diagnosis and Workup of Patients for Osteoporosis or Osteopenia (Low Bone Mass)

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1. Introduction

The rate of screening and treatment for osteoporosis remains low. Osteoporosis affects approximately 200 million women worldwide and two thirds of women 80 or older have this disease. It causes more than 1.6 million hip fractures worldwide. (International Osteoporosis Foundation, 2006) In the United States, more than 10 million Americans have osteoporosis and 3.6 million have low bone mineral density of the hip. In 2005 in the United States, there were more than 2 million osteoporotic fractures in men and women. These fractures caused more than 432,000 hospitalist admissions, 2.5 million physician office visits and about 180,000 nursing home admissions yearly in the United States. In 2005, the cost of osteoporotic fractures was approximately 17 billion dollars. (U.S. Department of Health and Human Services, 2004) Osteoporosis is responsible for approximately 90% of all spine and hip fractures in Caucasian females age 65-84 in the United States.

Fifty percent of women and twenty-five percent of men greater than age 50 will experience an osteoporotic fracture in their remaining lifetime. (National Osteoporosis Foundation, Feb. 2008) Less that 5 percent of patients with a clinical low trauma fragility fracture are referred for medical evaluation and treatment. (Bonura, 2009) In 2005, a population based study reported that the proportion of women screened and treated for osteoporosis after a fragility fracture was 10.2 percent and 12.9 percent respectfully. In males, studies indicate that a low proportion of men are evaluated for osteoporosis or receive anti-resorptive therapy after a fragility fracture. (Feldstein, 2005) Most patients with hip fractures receive no pharmacologic treatment for osteoporosis.

Physician frequently fail to diagnose and treat osteoporosis, even in the elderly patients who have suffered a fractures. (Bonura, 2009) Less then 20 percent of women with wrist fractures are screened for osteoporosis, and only 12.9 percent are treated for osteoporosis after a facture. (Cuddihy, 2002) In a clinical study the majority of patients with clinical vertebral fractures (80 percent) did not receive osteoporosis therapy. (Lindsay, 2005) A prior osteoporotic fracture increases the risk of future fractures. A forearm fracture is associated with a two fold increased risk of fractures. Radiographic vertebral fractures are associated with a higher risk of subsequent hip and other fractures. In the year following a vertebral fracture, 26 percent of patients will fracture a hip, pelvis, vertebrae, wrist, humerus, or leg. (Klotzbuecher, 2000)

Following a hip fracture in women, the mortality ranges between 20-24% within one year. 25% of patients are admitted to a long term healthcare facility and only 40% are able to obtain their pre-fracture level of independence. Worldwide, one-third of hip fractures occur

in men and more men than women die after a hip fracture with a mortality rate of about 37.5%. (Jiang, 2005) The most common of all the osteoporotic fractures are vertebral fractures. They are usually painless, but can cause back pain, height loss, deformity, reeducated respiratory function, disability, and a reduced quality of life. There is also an increase in mortality in women of about 23% over 8 years and they can cause an increase in future vertebral and non vertebral fractures. (Kado, 1999)

Prior Fracture	Relative Risk of Future Fractures		
	Wrist	Vertebral	Hip
Wrist	3.3	1.7	1.9
Vertebral	1.4	4.4	2.3
Hip	Not Available	2.5	2.3

Table 1. Prior Fracture as a Predictor of Fracture Risk (Klotzbuecher, 2000))

2. Evaluation

Postmenopausal women and men after age 50 should be evaluated for their risk factors for osteoporosis and fracture, and their risk factors of falling. This evaluation includes a history and a physical exam, including a height measurement and, if necessary diagnostic testing. Several clinical risk factors for osteoporosis have been indentified and should be evaluated. Non-modifiable risk factors include advancing age, female sex, Asian or Caucasian ethnicity, history of a fracture as an adult, family history of a fracture in a first degree relative (maternal or paternal history of a hip fracture) and rheumatoid arthritis. Modifiable risk factors comprise low body weight, hormone deficiency, long term use of medications that affect bone homeostasis (e.g., glucocorticoids), causes of secondary osteoporosis, smoking, excessive alcohol consumption, an inactive lifestyle, and a lifetime diet low in calcium and vitamin D. The more risk factor that a patient has, the greater the risk of a fracture. (Bonura, 2009)

The most important of all these risk factors are age (65 or greater in women and 70 or greater in men) and the occurrence of a low trauma fracture after age 40. Other risk factors of importance are bone mineral density, genetics, menopause, BMI, and lifestyle.

As a woman ages, their fracture risk increases. After age 50, their fracture risk doubles every 7 or 8 years. The average age of a hip fracture in women is 82 years and in men 50% of their hip fractures occur before age 80. Vertebral fractures usually occur in women and men in their seventies. (Chang, 2004) A prior osteoporotic fracture increases the risk of future osteoporotic fracture risk. If a postmenopausal female sustains an osteoporotic fracture, she has approximately a two fold increase of sustaining another osteoporotic fracture in her lifetime.

Bone mineral density also is a risk factor for future fractures. Bone mineral density affects fracture risk. The lower the bone mineral density (BMD), the higher the risk for fractures. A decrease of 1 standard deviation of bone mineral density (BMD) represents a 10-12% decrease in BMD and can increase fracture risk by 1.5 to 2.6 times. (Marshall, 1996) Genetics plays a role in osteoporosis and future fracture risk. Daughters of women who have had an osteoporotic fracture, and daughters of first degree relatives (mother or sisters) or have osteoporosis have lower bone mineral density (BMD) for their age. Also a history of an

Lifestyle Factors		
Low calcium intake	Vitamin D insufficiency	Excess vitamin A
High caffeine intake	High salt intake	Aluminum (in antacids)
Alcohol (3 or more drinks/d)	Inadequate physical activity	Immobilization
Smoking (active or passive)	Falling	Thinness
Hypogonadal States		
Androgen insensitivity	Hyperprolactinemia	Premature ovarian failure
Anorexia nervosa and bulimia	Panhypopituitarism	Turner's & Klinefelter's syndromes
Athletic amenorrhea		
Endocrine Disorders		
Adrenal insufficiency	Diabetes mellitus	Thyrotoxicosis
Cushing's syndrome	Hyperparathyroidism	
Gastrointestinal Disorders		
Celiac disease	Inflammatory bowel disease	Pancreatic disease
Gastric bypass	Malabsorption	Primary biliary cirrhosis
GI surgery		
Genetic Factors		
Cystic fibrosis	Homocystinuria	Osteogenesis imperfecta
Ehlers-Danlos	Hypophosphatasia	Parental history of hip fracture
Gaucher's disease	Idiopathic hypercalciuria	Porphyria
Glycogen storage diseases	Marfan syndrome	Riley-Day syndrome
Hemochromatosis	Menkes steely hair syndrome	
Hematologic Disorders		
Hemophilia	Multiple myeloma	Systemic mastocytosis
Leukemia and lymphomas	Sickle cell diseases	Thalassemia
Rheumatic and Autoimmune Diseases		
Ankylosing spondylitis	Lupus	Rheumatoid arthritis
Miscellaneous Conditions and Diseases		
Alcoholism	Emphysema	Muscular dystrophy
Amlyloidosis	End stage renal disease	Parenteral nutrition
Chronic metabolic acidosis	Epilepsy	Post-transplant bone disease
Congestive heart failure	Idiopathic scoliosis	Prior fracture as an adult
Depression	Multiple sclerosis	Sarcoidosis
Medications		
Anticoagulants (heparin)	Cancer chemotherapeutic drugs	Glucocorticoids (≥ 5 mg/d of prednisone or equivalent for ≥ 3 mo)
Anticonvulsants	Cyclosporine A and tacrolimus	Gonadotropin releasing hormone agonists
Aromatase inhibitors	Depo-medroxyprogesterone	Lithium
Barbiturates		

Table 2. Conditions, Diseases and Medications That Cause or Contribute to Osteoporosis and Fractures (National Osteoporosis Foundation, 2008)

osteoporotic fracture in a first degree relative increases an individual's risk of osteoporotic fractures. (Seaman, 1989) During the late menopausal transition (2-3 years before menopause) and during menopause, there is an increase in bone resorption due to a decrease in estrogen production. Women lose approximately 2% of BMD annually for about 5 years during menopause. After which they lose 1-2% per year. They can lose 10.5% in their spine and 5.3% in their hip over this 5-7 year period in this time. (Recker, 2000) In men bone loss increases after age 70 and it is more common in men who are deficient in testosterone or estradiol. (Fink, 2006)

If a woman is thin, a weight of less than 127 pounds, it is a risk factor for a low BMD and a high risk for fracture, A high BMI may be protective. In older women, low BMI is associated with a higher fracture risk. (Van Der Voort, 2001)

The lifestyle factors that are associated with low bone mass and fracture risk are cigarette smoking, alcoholism, poor nutrition, and lack of physical activity, There are also secondary causes of low bone mineral density, including medications, genetic disorders, and various disease states.

Also, in all menopausal women and men after age 50, their risk for falls should be evaluated. About one-third of men and women age 65 years of age or older fall each year. They should be questioned about their history of falls, muscle weakness, dizziness, difficulty walking, impaired vision, balance problems, or medications that affect balance (e.g., sedatives, narcotics, anti-hypertensives). Some of the medications that have the highest association with falls are the serotonin-reuptake inhibitors, antiarrhythmic drugs, tricyclic antidepressants, neuroleptic agents, benzodiazepines, and the anticonvulsants. (Leipzig, 1999) Ten percent of these falls result in hip fractures and 90% of hip fractures are due to falls. An excellent screening test is the "Get Up and Go" test. Have a senior patient get up from a chair, without using their arms, and then have them walk and observe for unsteadiness. (Mathias, 1986) The more number of risk factors of falling, the greater risk of falls.

Medical Risk Factors		
Age	Medications causing over-sedation (narcotic analgesics, anticonvulsants, psychotropics)	
Anxiety and agitation	Orthostatic hypotension	
Arrhythmias	Poor vision and use of bifocals	
Dehydration	Previous fall	
Depression	Reduced problem solving and mental acuity and diminished cognitive skills	
Female gender	Urgent urinary incontinence	
Impaired transfer and mobility	Vitamin D insufficiency [serum 25-hydroxyvitamin D (25(OH)D) < 30 ng/ml (75 nmol/L)]	
Malnutrition		
Environmental Risk Factors	**Neuro and Musculoskeletal Risk Factors**	**Other Risk Factors**
Lack of assistive devices in bathrooms	Kyphosis	Fear of falling
Loose throw rugs	Poor balance	
Low level lighting	Reduced proprioception	
Obstacles in the walking path	Weak muscles	
Slippery outdoor conditions		

Table 3. Risk Factors for Falls (National Osteoporosis Foundation, 2008)

During a patient's examination, height measurement may be useful to indicate occult vertebral compression fractures, which are indicative of osteoporosis. They are the most common osteoporotic fractures in postmenopausal women, and two-thirds of these fractures are not clinically recognized. (Cauley, 2007) The loss of height, kyphosis, and back pain may be signs of vertebral fractures. Normally after achieving maximal height, women can lose up to 1.0 – 1.5 inches (2.0 – 3.8 cm) of height as part of the normal aging process, due to degenerative arthritis and shrinkage of intervertebral disks. Height loss of greater than 1.5 inches (3.8 cm) increases the risk of a vertebral fracture. One must suspect a vertebral fracture in postmenopausal women with a historical height loss greater than 4 cm (1.6 in.) or a prospective height loss greater than 2 cm (0.8 in.). In men, a historical height loss greater than 6 cm (2.4 in.) or a prospective height loss greater than 3 cm (1.2 in.). Vertebral fractures are associated with an increase of vertebral and nonvertebral fractures in the future. Nineteen percent of patients who have a vertebral fracture will sustain another fracture within one year. (Laster, 2007) Across a range of BMD, prevalent vertebral fractures increase fracture risk by up to 12 times. Risk assessments based only on BMD may overestimate the risk of future fractures in patients without vertebral fractures and under estimate the risk of future fractures in patients with vertebral fractures. (Siris, 2007) When measuring height annually, it should be performed with a stadiometer or a wall mounted ruler. If there is a historical height loss of more than 1.5 inches (3.8 cm) in menopausal women or than 2.4 inches (6 cm) in men, an evaluation to rule out vertebral fractures should be performed. This can be accomplished by a vertebral fracture assessment (VFA) or by a lateral thoracolumbar radiograph. It is also important in patients who have acute or chronic back pain or kyphosis (to rule out vertebral fractures).

3. Bone mineral density assessment

The diagnosis of osteoporosis is made by BMD. The decision to assess bone density should be based on the skeletal health and risk profile of the individualized patient. Table 4 and 5 summarizes the Internal Society of Clinical Densitometry and the North American Menopausal Society indications for Bone Mineral Density (BMD).

Indications for Bone Mineral Density (BMD) Testing – ISCD	
Women aged 65 and older	Adults with a disease or condition associated with low bone mass or bone loss
Postmenopausal women under age 65 with risk factors for fracture	Adults taking medications associated with low bone mass or bone loss
Women during the menopausal transition with clinical risk factors for fracture, such as low body weight, prior fracture or high-risk medication use	Anyone being considered for pharmacologic therapy
Men aged 70 and older	Anyone being treated, to monitor treatment effect
Men under age 70 with clinical risk factors for fracture	Anyone not receiving therapy in whom evidence of bone loss would lead to treatment
Adults with a fragility fracture	Women discontinuing estrogen should be considered for bone density testing according to the indications listed above

Table 4. Indications for Bone Mineral Density (BMD) Testing - ISCD (Baim, 2008))

Indications for Bone Mineral Density (BMD) Testing - NAMS	
BMD Should Be Measured in the Following Populations:	Testing should be considered for postmenopausal women age 50 and over when one or more of the following risk factors for fracture have been identified
• All women age 65 and over regardless of clinical risk factors	• Fracture (other than skull, facial bone, ankle, finger, and toe) after menopause
• Postmenopausal women with medical causes of bone loss (eg, steroid use, hyperparathyroidism), regardless of age	• Thinness (body weight < 127 lbs. [57.7 kg] or BMI < 21 kg/m^2)
• Post menopausal women age 50 and over with additional risk factors (see below)	• History of hip fracture in a parent
• Postmenopausal women with a fragility fracture (eg, fracture from a fall from standing height)	• Current smoker
	• Rheumatoid arthritis
	• Alcohol intake of more than two units per day (one unit is 12 oz. of beer, 4 oz. of wine, or 1 oz. of liquor)

Table 5. Indications for Bone Mineral Density (BMD) Testing of the North American Menopause Society (NAMS) (North American Menopause Society, 2010)

There are many techniques that measure BMD. The gold standard in diagnosis of osteoporosis is made by central DXA using dual energy absorptiometry. Using two different x-ray energies, a DXA device can record attenuation profiles at two different photon energies. At low energy (30-50 keV) bone attenuation is greater than soft tissue attenuation; where as high energy (greater than 70 keV) bone attenuation is similar to soft tissues attenuation. Thus, two types of tissue are distinguished: bone (hydroxyapatite) and soft tissue (everything else). (International Society of Clinical Densitometry, 2010) DXA measures areal BMD. The results are reported as grams of mineral per square centimeter (g/cm^2).

The skeletal sites that are measured with central DXA are both the PA spine (L_1 – L_4) and the hip (femoral neck or total proximal femur of either hip). In certain circumstances when a hip or a spine cannot be measured then a forearm (33% radius or one third radius of the non dominant arm) should be measured (e.g., patients who are obese and whose weight is above the limit of the DXA table). Results of DXA are reported as a comparison of two norms. A T-score uses a Caucasian female 20-29 NHANES III database, for women of all ethnic groups. In men, a Caucasian male 20-29 NHANES III database is used in all ethnic groups. The difference between the patient's score is expressed in standard deviation above or below the norm. Also, a Z-score will be generated. The patient's BMD is compared to individuals of the same age, sex, and ethnicity. Z-scores should be population specific where adequate reference data exists (International Society for Clinical Densitometry, 2009).

The lower the BMD (T-score) the higher the fracture risk. A decrease of 1 standard deviation represents a 10 - 12% decrease in BMD and an increase in fracture risk by a factor of 1.5 to 2.6, depending on fracture type. The risks of spine and hip fracture increase 2.3 fold and 2.6 fold respectively, for each decrease of 1 standard deviation at the spine and hip. A Z-score of -2.0 or lower is defined as below expected range for age. A Z-score above -2.0 is within the expected range for age.

Peripheral Dual Energy X-Ray Absorptiometry (pDXA) measures areal bone density of the forearm, finger, or heel. It can indentify individuals at risk for fracture but this modality cannot be used for the diagnosis of osteoporosis or for follow up of patients. The measurement of peripheral sites are useful only in screening patients for the need of a central DXA, they are not useful for follow up in patients or in patients taking medications for osteoporosis. (Recker, 2000) Quantitative Computed Tomography (QCT) can measure spinal BMD and can predict vertebral fractures in women, but there is a lack of evidence of a prediction of vertebral fractures in men. There is no evidence that spine QCT can predict hip fractures in women or men. Peripheral Quantitative Computed Tomography (pQCT) of the ultra distal radius can predict hip fracture risk, but not spine fracture risk in postmenopausal women. Quantitative Ultrasound (QUS) can predict fragility fractures in postmenopausal women (hip and vertebral) and men over age 65 (hip and nonvertebral fractures). (Baim, 2008)

According to the World Health Organization (WHO) only the measurement of BMD by central DXA can be used for the diagnosis of osteoporosis, the follow up of individuals and the monitoring of treatment efficacy. (National Osteoporosis Foundation, 2008)

The WHO has defined low bone mass (Osteopenia) as a BMD between -1.0 and 2.5 SD below the normal for young healthy adults of the same sex (T-score < 1.0 and > -2.5), osteoporosis by a BMD of -2.5 SD or below (T-score ≤ -2.5) and severe osteoporosis with a T-score of ≤ -2.5 and a fragility fracture. (World Health Organization, 2003)

The World Health Organization has established the following definitions based on BMD measurement at the spine, hip, or forearm by DXA devices:		
Normal	**Low Bone Mass (Osteopenia)**	**Osteoporosis**
BMD is within 1 SD of a "young normal" adult (T-score at -1.0 and above.	BMD is between 1.0 and 2.5 SD below that of a "young normal" adult (T-score between -1.0 and -2.5).	BMD is 2.5 SD or more below that of a "young normal" adult (T-score at or below -2.5). Patients in this group who have already experienced one or more fracture are deemed to have severe or "established" osteoporosis."

Note: Although these definitions are necessary to establish the presence of osteoporosis, they should not be used as the sole determinant of treatment decisions.

Table 6. Defining Osteoporosis by BMD (Kanis, 1994)

In postmenopausal women and men age 50 and older, T-scores are preferred, using the WHO criteria. In women prior to menopause and in males younger than age 50, Z-scores, not T-scores are preferred. The WHO diagnostic criteria may be applied to women in the menopausal transition with risk factors for osteoporosis.

At the time of a central DXA, a vertebral fracture assessment (VFA) can be performed. It is a densitometric spine imaging than can detect vertebral fractures. (Schousboe, 2008) Most vertebral fractures are asymptomatic and if present can increase an individual's future risk of fracture. A Vertebral Fracture Assessment (VFA) is a diagnostic method in which low intensity or dual x-ray absorptiometry is used to examine the lateral spine (T_4-L_4), thereby identifying vertebral fractures. (Leipzig, 1999) There is much less radiation with a VFA in comparison to a lateral spine x-ray (3μSV for VFA versus 600μSV for a radiograph).

According to the International Society of Clinical Densitometry a vertebral fracture assessment should be performed in the following circumstances:

Consider VFA when results may influence management.	
Postmenopausal women with low bone mass (Osteopenia) by BMD criteria PLUS any one of the following:	**Men with low bone bass (Osteopenia) by BMD criteria, PLUS any one of the following:**
• Age greater than or equal to 70 years	• Age 80 years or older
• Historical height loss greater than 4 cm (1.6 in.)	• Historical height loss greater than 6 cm (2.4 in.)
• Prospective height loss greater than 2 cm (0.8 in.)	• Prospective height loss greater than 3 cm (1.2 in.)
• Self-reported vertebral fracture (not previously documented)	• Self-reported vertebral fracture (not previously documented)
• Two or more of the following:	• Two or more of the following:
• Age 60 to 69 years	• Age 70 to 79 years
• Self-reported prior non-vertebral fracture	• Self-reported prior non-vertebral fracture
• Historical height loss of 2 to 4 cm	• Historical height loss of 3 to 6 cm
• Chronic systemic diseases associated with increased risk of vertebral fractures (e.g., moderate to severe COPD or COAD, seropositive rheumatoid arthritis, Crohn's disease)	• Chronic systemic diseases associated with increased risk of vertebral fractures (e.g., moderate to several COPD or COAD, seropositive rheumatoid arthritis, Crohn's disease
	On pharmacologic androgen deprivation therapy or following orchiectomy
Women or men on Chronic Glucocorticoid therapy (equivalent to 5 mg or more of prednisone daily for three (3) months or longer).	**Postmenopausal women or men with osteoporosis by BMD criteria, if documentation of one or more vertebral fractures will alter clinical management.**

Table 7. Indications for VFA (Schousboe, 2008)

The VFA is interpreted using a semi-quantitative visual inspection with assignment of fracture grade by the radiologist or the clinician. Using Genant's method, the clinician determines if a vertebra is fractured or normal. The thoracic and lumbar spine is scanned for deformities and height loss exceeding twenty percent in the anterior, middle, or posterior dimensions. What the clinician determines visually using the semiquantitative method of Genant are morphological changes in the vertebrae. Clinicians should look for end plate deformities (horizontal edges), lack of parallelism of the end plates, buckling of the cortices (vertical edges) and loss of vertical continuity with the adjacent vertebrae. Clinicians then grade the fracture deformity. If a fracture is suspected, it is compared to a standard:

- Grade 1 (mild fracture) height loss 20-25%
- Grade 2 (moderate) height loss of 25-40%
- Grade 3 (severe) height loss of > 40%

Depending on where deformities are present in the vertebra, fractures are classified as wedge (loss of anterior height), crush (loss of posterior height) and biconcave (loss of middle height). (Genant, 1993)

In a study comparing VFA to spine radiographs, VFA had a sensitivity of 95% to detect vertebral fractures indentified by spine radiographs and a specificity of 82% to exclude fractures not visualized in a radiograph. (Vokes, 2003)

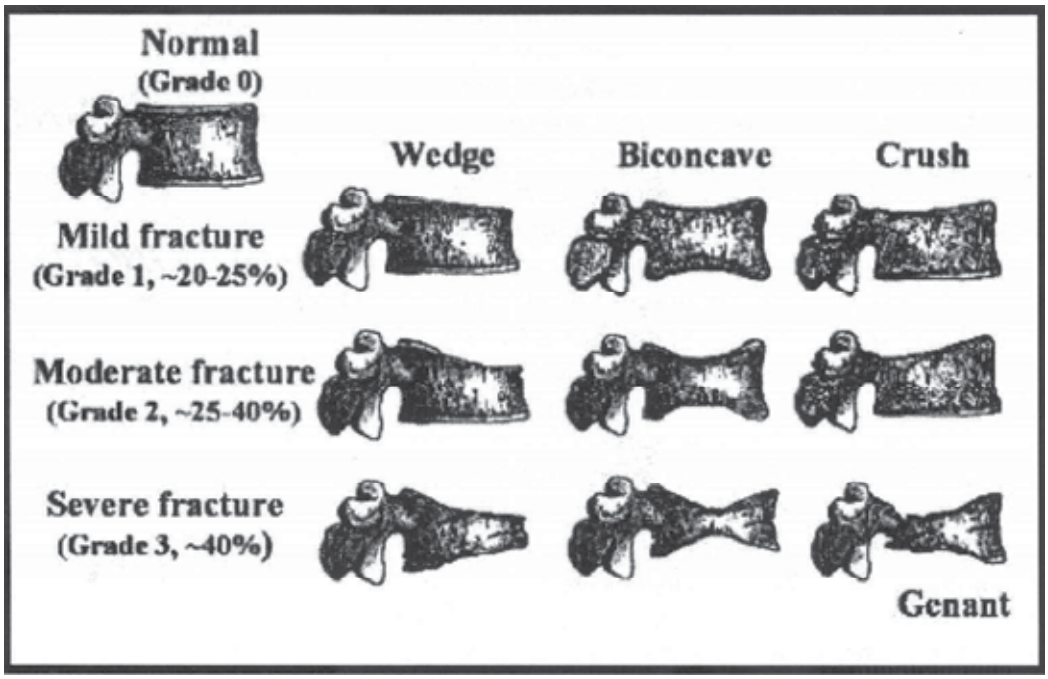

Fig. 1. Genant's Semiquantitative Analysis Grading of Fracture Deformity (Genant, 1993)

4. Follow up bone mineral density testing

Follow up DXA testing by central DXA should be performed every 2-5 years in untreated menopausal women and in men age 70 or older. In patients who are receiving osteoporosis therapy, BMD testing should be performed every 1-2 years. (Van Der Voort, 2001) In order to determine if the change in BMD over time is a real biological change and not to due to chance, a precision study must be performed. Each DXA facility should determine its precision error and calculate the least significant change (LSC), within a 95% statistical confidence. The precision error that is supplied by the manufacturer should not be used.

To perform a precision analysis, the technologist measures 15 patients 3 times or 30 patients 2 times, repositioning the patient after each scan. They then calculate the root mean square stand deviation and obtain the LSC at 95% confidence. This is a real biological change over time and is not due to chance. The minimum acceptable precision for an individual technologist is: lumbar spine 1.9% (LSC = 5.3%), total hip 1.8% (LSC = 5.0%), and femoral neck 2.5% (LSC = 6.9%). (International Society for Clinical Densitometry, 2007)

5. Evaluation for treatment

Within one year of a hip fracture 10-20% of patients die, 20% are placed in nursing homes, and only 40% regain independent functioning. (U.S. Department of Health and Human

Services, 2004) There are more low-trauma fractures in patients with low bone density (Osteopenia) than in those with a DXA diagnosis of osteoporosis. This occurs because there are more individuals with Osteopenia than osteoporosis. (Khosla, 2007) In the study of osteoporotic fractures (SOF), 54% of women with hip fractures did not have osteoporosis according to their BMD results. (Wainwright, 2005) Therefore, it is important to identify and treat individuals who have Osteopenia (low bone density) who have the highest chance of a fracture. Not all patients with low bone mass will fracture.

6. FRAX assessment tool

The WHO sponsored the development of the FRAX assessment tool to indentify which individuals with low bone mineral density have the greatest chance of fracture and which patients need to be treated. (Kanis, 2008) It identifies which patients, who have Osteopenia (low bone lass) who have a higher fracture risk and need to be treated. (Internal Society for Bone Densitometry Course) FRAX is based on an analysis of approximately 60,000 patients that were studied in Europe, North American, Asia, and Australia. The FRAX tool is for untreated individuals with low bone density and indentifies which individuals are at the highest risk of fracture. The NOF recommends the FRAX tool for untreated postmenopausal and men 50 or more years of age with a T-score in the osteopenic range (low bone mass). FRAX predicts the 10 year probability for hip fracture and for major osteoporotic fractures (hip, proximal humerus, distal forearm, and clinical vertebral fractures). FRAX uses clinical risk factors with or without femoral neck BMD. Economic modeling was performed to indentify the 10 year hip fracture risks above which is cost effective, from the societal perspective, to treat with pharmacological agents.

NOF criteria for using FRAX to assist with treatment decisions are:

a. An untreated postmenopausal women or a man age 50 or older
b. With low bone mass (T-score between -1.0 and -2.5)
c. With no prior hip or vertebral fracture (clinical or morphometric)
d. An evaluable hip for DXA study

Examples of "untreated" patients include:

a. No ET/HT or estrogen agonist/antagonist (SERM) for the past one year
b. No calcitonin for the past one year
c. No PTH for the past one year
d. No denosumab for the past one year
e. No bisphosphonate for the past two years (unless it is an oral taken for < 2 months)

This model uses femoral neck BMD but, in women, if femoral neck BMD is unavailable, total hip BMD may be used. In men, only femoral neck BMD can be used in FRAX. (World Health Organization, 2003) Spine of peripheral BMD measurements should not be used in FRAX. (Kanis) There are multiple limitations of FRAX. It does not consider the other risk factors for fracture. These include a history of falls, patients with clinical vertebral fractures, doses of glucocorticoids, exposure dose of alcohol, nicotine, drugs which lower BMD (anticonvulsants, anticoagulants, antineoplastics, antiestrogenic or antiandrogenic agents), parental history of non hip fractures and lumbar spine BMD. This tool only recognizes a hip fracture of a parent. Spinal fractures of a parent due to osteoporosis also may increase fractures risk in the offspring. The therapeutic thresholds that are proposed in the FRAX tool are for clinical guidance and are not rules. They do not preclude clinicians from considering intervention strategies in patients who do not have osteoporosis, nor should they mandate treatment in patients with osteopenia. The decision to treat a patient must be made on a case by case basis.

Risk Factor	Type of Variable	Description
Age	Continuous	40-90 years. For ages below or above this range, the model computes fracture risks as of age 40 or 90.
Sex	Male/female	Model validated for men and women.
Weight	Continuous	Expressed in kilograms.
Height	Continuous	Expressed in centimeters. The modal calculates body mass index and uses it as a continuous variable.
Previous fracture	Yes/no	Includes adult fractures occurring spontaneously or with low trauma (e.g., osteoporosis-related fractures), including morphometric vertebral fractures.
Parental hip fracture	Yes/no	Any hip fracture affecting either parent.
Current smoking	Yes/no	Dose-dependence of fracture risk has been observed but is not reflected in the model.
Oral glucocorticoid use	Yes/no	Present or past exposure to doses equivalent to 5 mg/d prednisolone for ≥ 3 months. Dose-dependence of fracture risk has been observed but is not reflected in the model.
Rheumatoid arthritis	Yes/no	Only a confirmed diagnosis of rheumatoid arthritis should be scored.
Secondary osteoporosis	Yes/no	Secondary osteoporosis occurs in the presence of conditions including, but not limited to, insulin-dependent diabetes, adult osteogenesis imperfecta, uncontrolled hyperthyroidism, menopause at 45 years, hypogonadism, chronic malnutrition/malabsorption, or chronic liver disease.
Alcohol ≥ 3 units daily	Yes/no	1 unit is 285 mL beer, 120 mL wine, 60 mL aperitif, or 30 mL distilled spirits. Dose-dependence of fracture risk has been observed but is not reflected in the model.
Femoral neck BMD	Continuous	Enter BMD value and select DXA machine model used to obtain it, or enter T-score. Risk estimates can also be produced without BMD. If only total hip BMD is available, that can be used. Spinal or peripheral BMD are not to be used.
Clinical vertebral fractures and multiple osteoporosis-related fractures confer additional risk beyond what the model calculates; clinical judgment should be used.		
For a "yes" answer to smoking, alcohol, or glucocorticoid use, the model assumes average levels of exposure. With high exposures, facture risk may increase more than the model accounts for, and clinical judgment should be used.		
When a femoral neck T-score is entered into the online FRAX model, the secondary osteoporosis button becomes inactivated.		

Table 8. Risk Factors Included in the FRAX Algorithm (Siris, 2010)

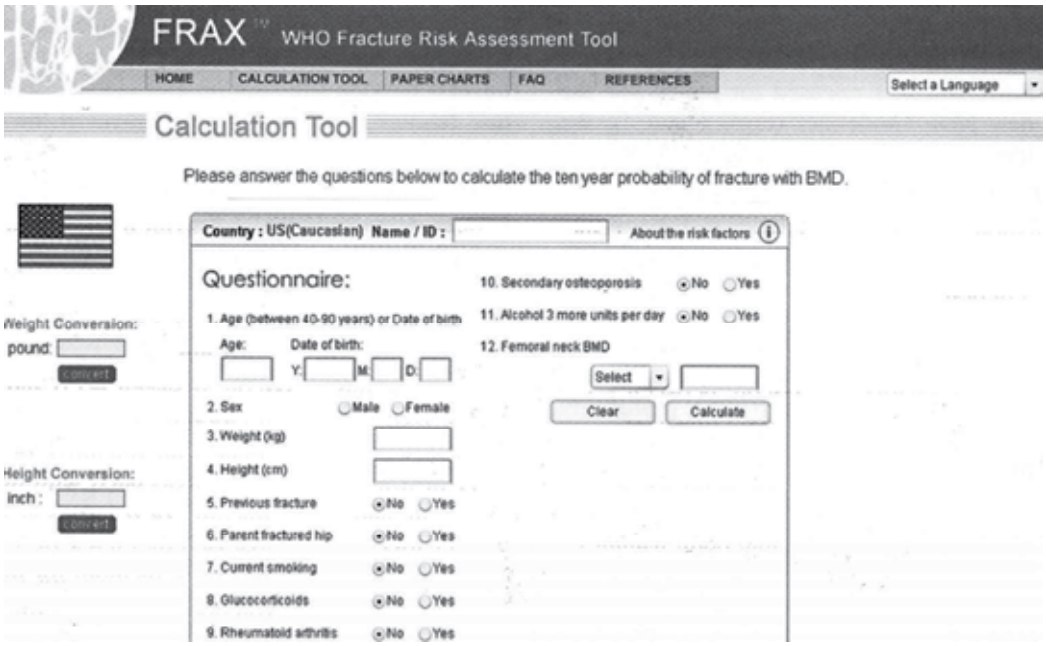

Fig. 2. FRAX Calculation Tool

7. Whom should we treat: NOF guidelines

Consider FDA approved medical therapies for patients with:

- A hip or vertebral (clinical or morphometric) fracture
- Osteoporosis diagnosed by a BMD T-score ≤ -2.5 (femoral neck or spine) after appropriate evaluation to exclude secondary causes of osteoporosis
- Low bone density (a BMD score between -1 and -2.5 at the femoral neck or spine) and a FRAX 10 year probability of hip fracture ≥ 3% or a major osteoporotic fracture (hip, wrist, proximal humerus, clinical vertebral fracture) of ≥ 20%

Physicians should use clinical judgment to treat patients at lower FRAX risk levels if additional risk factors for fracture are present.

Before developing a management plan, the clinician should rule out secondary causes of osteoporosis or bone loss. About 20% of postmenopausal women with osteoporosis and 40% of men with osteoporosis have a secondary cause that can be indentified and treated. (Fitzpatrick, 2002) Secondary osteoporosis can result from a variety of medical conditions including endocrine, hematopoietic or nutritional disorders, and vitamin D deficiency. Diseases such as celiac (malabsorption), liver and renal diseases, the use of glucocorticoids, aromatase inhibitors, antiandrogen therapy (GNRH) and chemotherapy can cause bone loss. These secondary causes must be ruled out before staring pharmacological therapy.

Some of the routine tests would be a complete blood count, serum levels of calcium and phosphate, 25-hydroxyvitamin D, bone specific alkaline phosphatase, creatinine and a 24-hour urine for calcium. Some of the specialized tests may include a thyroid stimulating hormone (TSH), serum levels of parathyroid hormone (PTH) to screen for hyperparathyroidism, a serum protein electrophoresis to indentify abnormal protein produced by multiple myeloma and antitissue transglutaminase antibodies for celiac disease. (Hodgson, 2003)

Test	Diagnostic Result	Possible Secondary Cause
Completed blood cell count	Anemia	Multiple myeloma
Serum calcium	Elevated	Hyperparathyroidism
	Low	Vitamin D deficiency, Gastrointestinal (GI) malabsorption
Serum phosphate	Elevated	Renal failure
	Low	Hyperparathyroidism
Serum 25-hydroxyvitamin D	Low	Undersupplemenataion, GI malabsorption, celiac disease
Serum albumin	Used to interpret serum calcium, nutritional deficiencies	
Serum alkaline phosphatase	Elevated	Vitamin D deficiency, GI malabsorption, hyperparathyroidism, Paget's disease, liver/biliary disease
Urinary calcium excretion	Elevated	Renal calcium leak, multiple myeloma, metastatic cancer involving bone, hyperparathyroidism, hyperthyroidism
	Low	GI malabsorption, inadequate intake of calcium and vitamin D
Thyroid-stimulating hormone (TSH)	Low	Hyperthyroidism (causes excess bone turnover)
	High	Hypothyroidism
Serum protein electrophoresis	Monoclonal band	Multiple myeloma
Tissue transglutaminase antibody (gluten enteropathy)	Elevated	Predictive of celiac disease
Creatinine	Elevated	Renal osteodystrophy, possible contraindication to bisphosphonates
PTH	Elevated	Hyperparathyroidism, vitamin D deficiency

Table 9. Laboratory Tests for Osteoporosis Evaluation (North American Menopause Society, 2010)

8. Bone turnover markers

Bone turnover markers are proteins that are produced by the activity of osteoclasts and by osteoblasts. The resorption markers of osteoclastic activity are the breakdown products of type I collagen (N-telopeptides, C-telopeptides, deoxypyridinolone). There are markers of osteoblastic synthesis of bone matrix (bone-specific alkaline phosphatates, osteocalcin, procollagen type I N-terminal propetide).

Suppression of biochemical markers of bone turnover occur 3-6 months of specific antiresorptive therapies and they increase after 1-3 months of anabolic therapies. The NOF recommends a baseline and a repeat bone resorption marker after initiation of therapy as a method of monitoring the therapeutic effect of an antiresportive agent.
The changes of bone turnover markers occur more rapidly than changes in BMD and they can also predict patient compliance or poor response to antiresorptive therapy. There is a high degree of biological and analytical variability in the measurement of biochemical markers. This variability can be reduced by obtaining samples in the early morning after an overnight fast. (National Osteoporosis Foundation, 2008)

9. Conclusion

In all senior men and in all postmenopausal women a history should be obtained to evaluate a patient's risk factors for osteoporosis and for fractures. As part of a patient's physical exam, a height measurement should be performed. This can indentify when there is a significant height loss or an asymptomatic vertebral fracture. When necessary, a central DXA and sometimes a Vertebral Fracture Assessment (VFA) may be performed. Clinicians should estimate a patient's 10-year probability of a hip or a major osteoporotic related fracture using FRAX. We should use the WHO criteria to determine who we should treat and which individuals whom we should not treat. Clinicians also must perform a laboratory workup on patients, to rule our secondary causes of osteoporosis, before starting a pharmacological treatment regimen.

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7

Evolutionary Pathways of Diagnosis in Osteoporosis

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1. Introduction

Osteoporosis was formally identified as a disease by a group of World Health Organization (WHO) experts in 1994 resulting in publication of "Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis" (WHO Technical Report Series, 1994).
Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. Bone strength reflects the integration of two main features: bone density and bone quality. Bone density is expressed as grams of mineral per area or volume and in any given individual is determined by peak bone mass and amount of bone loss. Bone quality refers to architecture, turnover, damage accumulation and mineralization (NIH Consensus, 2001).
Osteoporosis occurs in all populations and at all ages and is a devastating disorder with significant physical, psychosocial and financial consequences. The WHO operationally defines osteoporosis as a bone density at least 2.5 standard deviations below the mean peak bone mass for healthy young adult white women, also referred to as a *T-score* of –2.5. Because of the difficulty in accurate measurement and standardization between instruments and sites, controversy exists among experts regarding the continued use of this diagnostic criterion. So different instruments have not the same performance in regard to a accurate bone density measurement.
The aims of this chapter are stated in table 1.

1.	Identify the technique, safety and limitations of dual energy X-ray absorptiometry (DEXA or DXA) scanning.
2.	Explain the value of utilizing bone densitometry to assess and monitor fracture risk.
3.	Incorporate clinical risk factors that predict future fracture.
4.	Explain the value of identifying the different components that make up the bone metabolism.
5.	Open new tracks for diagnosis of osteoporosis

Table 1. Statement of objectives

In the evolutionary pathways of diagnostics in bone loss the osteoporosis diagnosis is often performed by measuring bone mineral density (BMD) that measures the amount of calcium in different regions of the skeleton as femur neck or/and 1-4 lumbar vertebrae .

In establishing diagnosis of osteoporosis three parameters should be considered as stated in table 2.

1. The diagnosis of osteoporosis. 2. The diagnosis of bone metabolism components.

Table 2. Statement of objectives

2. Osteoporosis and fracture risk: Monitoring and assessment

Several methods are available to measure BMD. In general, the lower bone density the greater osteoporotic fracture risk. Unfortunately osteoporosis frequently remains undiagnosed until a fracture occurs. BMD methods involve DEXA or quantitative computer tomography scans (Osteo CT or QCT) of bones in the spine or femur. The most widely used technique is DXA.

2.1 Technique, safety and limitations of DXA scanning

Bone densitometry is the *gold standard method* for measuring BMD. Bone densitometry is the method used to determine the drug efficacy in recent large clinical trials and to characterize fracture risk in large epidemiological studies. DXA, previously DEXA, is a method of measuring BMD. A DXA scan uses low energy X-rays. A machine sends X-rays from two different sources through the bone being tested. Bone blocks a certain amount of the X-rays. The more dense the bone is, the fewer X-rays get through to the detector. By using two different X-ray sources rather than one it greatly improves the accuracy in measuring the bone density. The amount of X-rays that comes through the bone from each of the two X-ray sources is measured by a detector. This information is sent to a computer which calculates a score of the average density of the bone. A low score indicates that the bone is less dense than it should be, some material of the bone has been lost, and is more prone to fracture.

Older methods such as single photon absorptiometry(SPA) do not predict hip fractures as well as DXA.

But currently there is no accurate measure of overall bone strength. Osteoporosis is related to decreased bone strength, which encompasses both BMD and bone quality. Notwithstanding BMD assessed by DXA remains the *gold standard* for the diagnosis of osteoporosis.

DEXA is the most widely available method of bone densitometry. The measurement of BMD by DEXA has served as a fit surrogate for the measurement of bone strength and accounts for approximately 70 percent of bone strength, it was said. DEXA measures the BMD in the spine, hip or total body.

Based on the 1994 WHO report, osteoporosis is defined as a BMD value from at least -2.5 SD below the mean value of a young healthy population (T-score≤-2.5). Any bone can be affected, but of special concern are the fractures of the hip and spine.

Diagnosis of osteoporosis is generally on the basis of BMD assessment at the spine and proximal femur by DXA. Two X-ray beams with differing energy levels are targeted at the patient's bones. But, there are other variables in addition to age which are suggested to confound the interpretation of BMD as measured by DXA. One important confounding variable is bone size. DXA has been shown to overestimate the bone mineral density of taller

subjects and underestimate the bone mineral density of smaller subjects. This error is due to the way in which DXA calculates BMD. In DXA, bone mineral content, measured as the attenuation of the X-ray by the bones being scanned, is divided by the area, also measured by the machine, in the site being scanned.
Because of DXA calculates BMD using area (aBMD: areal Bone Mineral Density), it is not an accurate measurement of true bone mineral density, which is mass divided by a volume. In order to distinguish DXA BMD from volumetric bone-mineral density, researchers sometimes refer to DXA BMD as aBMD.
The National Osteoporosis Foundation's guidelines state that women over 65, younger post menopausal women who have any of the osteoporosis risk factors, as well as those with specific fractures should have this test. However, men are also at risk for osteoporosis as they age especially if they have some of the causes of osteopenia or osteoporosis

2.2 Other methods of osteoporosis diagnosis

The bone density test is performed using various methods. Some of these BMD tests are explained here briefly.

2.2.1 Quantitative ultrasound parameters

Quantitative Ultrasound (QUS) is the most basic bone density test performed. It can be the first step in order to diagnose any primary bone related problem. If the ultrasound test finds any defect in the bone density, then the DEXA test is recommended. QUS can be used to predict fracture risk, but it cannot be used for the diagnosis of osteoporosis or for monitoring the effects of treatment. Ultrasounds measure the BMD in the heel and uses sound waves of different frequencies through water or air, to perform the task. Bone density test is painless, fast and without harmful radiations. Ultrasounds are unable to detect complicated bone problems and hence there are other methods that are capable of detecting the more complicated ones.
Ultrasound axial transmission, a technique using propagation of ultrasound waves along the cortex of cortical bones, has been proposed as a diagnostic technique for the evaluation of fracture healing. Quantitative ultrasound parameters have been reported to be sensitive to callus changes during the regeneration process. The results suggest that the time of flight measured in axial transmission is affected by local changes of speed of sound induced by changes in local mineralization.

2.2.2 Quantitative computer tomography scan

Quantitative Computer Tomography Scan (QCT) is done to find true volumetric bone mineral content by measuring separately trabecular and three-dimensional cortical bone.
Image quality degradation due to subject motion is a common artefact affecting *in vivo* high-resolution peripheral quantitative computed tomography (HR-pQCT) of bones. These artefacts confound the accuracy and reproducibility of bone density, geometry, and cortical and trabecular structure measurements. Observer-based systems for grading image quality and criteria for deciding when to repeat an acquisition and post hoc data quality control remain highly subjective and non-standardized (Sodeab et al, 2011).
The QCT scan is a not so famous form of bone density test because it is expensive, utilizes a high amount of radiation and its accuracy is minimum.

The QCT measures BMD at spine or hip. Bone architecture, measured by CT, is a BMD-independent determinant of bone strength (Bauer & Link, 2009).
Because bone density can vary from one location in the body to another, a measurement taken at the heel usually is not as accurate a predictor of fracture risk as is a measurement taken at the spine or hip. That is why, if the test on a peripheral device is positive, DXA scan should be performed at the spine or hip to confirm the diagnosis. But what happen at the spine or hip is not what happen at the heel or wrist. So, the problem endures.

2.2.3 Bone fracture risk calculators

The fracture risk assessment tool (FRAX®) case finding algorithm has been developed to predict the 10-year risk of major and hip fractures based on clinical risk factors, with and without BMD. The Garvan fracture risk calculator is another tool that is available online to calculate the risk of fracture. The FORE Fracture Risk Calculator™ uses risk factors established by the W HO, such as alcohol use, family history of hip fractures, and certain chronic diseases.
FRAX and Garvan fracture risk calculators estimate the absolute risk of osteoporotic fractures. Garvan estimated higher absolute fracture risk than FRAX. None of the calculators provide better discrimination than models based on age and BMD, and their discriminative ability is only moderate, which may limit their clinical utility (Bolland et al., 2011).
The Framingham Osteoporosis Study, an ancillary study of the Framingham Heart Study, has contributed substantially to the understanding of risk factors for age-related bone loss and fractures in men and women. For the past fifteen years, this research program has been investigating a variety of risk factors for bone loss and fractures by assessing BMD using SPA, dual photon absorptiometry (DPA), DXA, QUS, and by ascertaining fracture incidence in the Framingham Study
The FRAX® tool has been developed by WHO to evaluate fracture risk of patients that integrate the risks associated with clinical risk factors as well as with or without BMD at the femoral neck. The FRAX® algorithms give the 10-year probability of fracture (Kanis et al., 2000).
The prediction of hip fracture and other osteoporotic fractures based on the assessment algorithms (FRAX™) which includes clinical risk factors alone, or the combination of clinical risk factors plus BMD is prediction, but Medicine is Medicine and future prediction is not Medicine and the important is not the statistics but if the human ill being who must be treated or not. In the evaluation of the FRAX and Garvan fracture risk calculators in older women it was found that Garvan calculator was well calibrated for osteoporotic fractures but overestimated hip fractures. FRAX with BMD underestimated osteoporotic and hip fractures. FRAX without BMD underestimated osteoporotic and overestimated hip fractures. In summary, none of the calculators provided better discrimination than models based on age and BMD, and their discriminative ability was only moderate, which may limit their clinical utility. The calibration varied, suggesting that the calculators should be validated in local cohorts before clinical use.
The probability is not certainty of fracture. It is statistics. That is science that deals with the collection, classification, analysis, and interpretation of numerical facts or data, and that, by use of mathematical theories of probability, imposes order and regularity on aggregates of more or less disparate elements. Is that the matter?. May be, but it is not medicine. And osteoporosis is a medical condition. And risk factors do not mean disease. The patient is the

patient and not one year probability. On the other hand "the FRAX® assessment does not tell you who to treat which remains a matter of clinical judgement. In many countries, guidelines are provided that are based on expert opinion and/or on health economic grounds", what the question remain to be wonder what to do with?. That supposes one first principles answer. Level D evidence-based medicine according to the standards of the UK National Health Service or lower level if any existed in the evidence-based medicine.

2.2.3.1 The Bayes' theorem

Bayes' theorem deals with the role of new information in revising probability estimates. The theorem assumes that the probability of a hypothesis (the posterior probability) is a function of new evidence (the likelihood) and previous knowledge (prior probability).
Specific chart reminders to physicians combined with mailed patient education substantially increased the levels of bone density testing and could potentially be used to improve osteoporosis screening in primary care. Bayesian hierarchical analysis makes it possible to assess practice-level interventions when few practices are randomized (Levy BT et al. 2009).
In probability theory and applications, Bayes' theorem shows how to determine inverse probabilities: knowing the conditional probability of B given A, what is the conditional probability of A given B? This can be done, but also involves the so-called prior or unconditional probabilities of A and B.
This theorem is named for Thomas Bayes and often called Bayes' law or Bayes' rule. Bayes' theorem expresses the conditional probability, or "posterior probability", of a hypothesis H (its probability after evidence E is observed) in terms of the "prior probability" of H, the prior probability of E, and the conditional probability of E given H. It implies that evidence has a confirming effect if it is more likely given H than given not-H. Bayes' theorem is valid in all common interpretations of probability, and it is commonly applied in science and engineering. However, there is disagreement among statisticians regarding the question whether it can be used to reduce all statistical questions to problems of inverse probability. Can competing scientific hypotheses be assigned prior probabilities?
The key idea is that the probability of an event A given an event B depends not only on the relationship between events A and B but also on the marginal probability of occurrence of each event.
As a formal theorem, Bayes' theorem is valid in all common interpretations of probability. However, frequentist and Bayesian interpretations disagree on how (and to what) probabilities are assigned. In the Bayesian interpretation, probabilities are rationally coherent degrees of belief, or a degree of belief in a proposition given a body of well-specified information. Bayes' theorem can then be understood as specifying how an ideally rational person responds to evidence. In the frequentist interpretation, probabilities are the frequencies of occurrence of random events as proportions of a whole. Though his name has become associated with subjective probability, Bayes himself interpreted the theorem in an objective sense.
Bayes' theorem is often more easy to apply, and to generalize, when expressed in terms of odds. It is then usually referred to as Bayes' rule, which is expressed in words as posterior odds equals prior odds times likelihood ratio. The term Bayes factor is often used instead of likelihood ratio.
In statistics, the use of Bayes factors is a Bayesian alternative to classical hypothesis testing. Bayesian model comparison is a method of model selection based on Bayes factors.
The adoption of Bayes' theorem has led to the development of Bayesian methods for data analysis. Bayesian methods have been defined as "the explicit use of external evidence in the

design, monitoring, analysis, interpretation and reporting" of studies (Spiegelhalter, 1999). The Bayesian approach to data analysis allows consideration of all possible sources of evidence in the determination of the posterior probability of an event. It is argued that this approach has more relevance to decision making than classical statistical inference, as it focuses on the transformation from initial knowledge to final opinion rather than on providing the "correct" inference.
In addition to its practical use in probability analysis, Bayes' theorem can be used as a normative model to assess how well people use empirical information to update the probability that a hypothesis is true.
The odds in favor of an event or a proposition are expressed as the ratio of a pair of integers, which is the ratio of the probability that an event will happen to the probability that it will not happen.
Frequency probability is the interpretation of probability that defines an event's probability as the limit of its relative frequency in a large number of trials. The development of the frequentist account was motivated by the problems and paradoxes of the previously dominant viewpoint, the classical interpretation. The shift from the classical view to the frequentist view represents a paradigm shift in the progression of statistical thought.
Frequentists talk about probabilities only when dealing with well-defined random experiments.The set of all possible outcomes of a random experiment is called the sample space of the experiment.
A paradigm is what members of a scientific community, and they alone, share. A paradigm shift (or revolutionary science) is, according to Thomas Kuhn in his influential book The Structure of Scientific Revolutions (1962), a change in the basic assumptions, or paradigms, within the ruling theory of science. It is in contrast to his idea of normal science. A proposition is true if it works. Thus, older occupants in motor-vehicle crashes are more likely to experience injury than younger occupants. Crash-injury data were used with Bayes' Theorem to estimate the conditional probability of AIS 3+ skeletal injury given that an occupant is osteoporotic for the injury to the head, spine, thorax, lower extremities, and upper extremities. It suggests that the increase in AIS 3+ injury risk with age for non-spine injuries is likely influenced by factors other than osteoporosis (Rupp et al., 2010).

2.2.4 The radiological assessment of vertebral osteoporosis

Vertebral fracture assessment (VFA) is recognized as the standard in fracture risk assessment. High definition instant vertebral assessment allows identifying spine fractures with one rapid, low dose, single energy image at double the resolution of previously available techniques. VFA differs from radiological detection of fractures, because VFA uses a lower radiation exposure and can detect only fractures, while traditional x-ray images can detect other bone and soft tissue abnormalities in addition to spinal fractures.

2.2.5 Some other methods

1. Morphometry. VFA may be referred to as DEXA or DXA or morphometric x-ray absorptiometry. Magnetic resonance imaging (MRI) is a new method of measuring bone density. MRI has made significant contributions to the diagnosis of acute hip joint disease in adults by enabling early differentiation between such conditions as idiopathic avascular femoral head necrosis, septic coxitis, degenerative disease, and tumors. MRI may provide information pertaining to bone density and structure as well as to occult fracture detection.

Quantitative methods such as morphometry or MRI have been developed over the past years and can be used to assess more precisely the features of vertebral fractures.

2. Single-energy X-ray absorptiometry (SXA) is a method of assessing bone mineral density using a single energy X-ray beam. This may be used to measure the wrist or heel bone density, but SXA is not used as often as DEXA. It is now widely considered inferior to dual-energy X-ray absorptiometry which uses a second energy beam to correct for absorption of X-ray energy by non-calcium containing tissues. Many previous studies of peripheral bone mineral density measurement for instance at the wrist, used SXA to assess bone mineral density.
3. Peripheral dual energy X-ray absorptiometry (PDEXA) is a type of DEXA test that measures the density of bones in the arms or legs, such as the wrist or a finger. It cannot measure the density of the bones most likely to break, such as the hip and spine. PDEXA is not as useful as DEXA for finding out how well medicine used to treat osteoporosis is working.
4. SPA is a method that uses a single-energy photon beam that is passed through bone and soft tissue to a detector. The amount of mineral in the path is then quantified.
5. Dual-photon absorptiometry (DPA) uses a photon beam that has two distinct energy peaks. One energy peak is absorbed more by the soft tissue. The other energy peak is absorbed more by bone. The soft-tissue component is subtracted to determine the BMD.
6. Radiographic absorptiometry (RA) uses an X-ray of the hand and a small metal wedge to calculate bone density. This is an approach that include different methods to significantly increase the proportion of eligible patients tested for low BMD, using a low-cost peripheral BMD system in the primary care physician's office or satellite facility to identify those patients who could receive further BMD assessment by central DXA.

2.3 The diagnosis of bone metabolism control

Bone metabolism control is performed inside and outside the bone. Through lab tests which may be carried out in blood and urine samples, bone metabolism becomes known. The results of these tests can help identify conditions that may be contributing to bone loss. The most common blood tests evaluate: blood calcium levels, blood vitamin D levels, liver function, kidney function tests: both creatinine and BUN are included on the common chemical profiles, thyroid function: TSH, T4, T3 tests , parathyroid hormone levels, estradiol levels in women, follicle stimulating hormone test to establish menopause status, testosterone levels in men, osteocalcin levels to measure bone formation.

A 24-hour urine collection can show if there is a problem with intestinal absorption of calcium (Ca) or leakage of calcium through the kidneys. Blood tests are done to check things such as blood chemistries, blood count, proteins, vitamin D level, thyroid function, and antibodies for celiac disease, a condition that may cause poor intestinal absorption of important nutrients. A simple urine specimen shows the bone metabolism or an important factor in determining bone density and bone strength. With this test, natural bone protein products such as N-telopeptide (NTX) are tested.

Dairy products constitute one of the most important types of functional food. And dairy products-calcium intake and its good intestinal absorption is basic. Renal Ca clearance is other parameter to be measured.

Essential hypertensive (EH) patients have a higher rate of urinary calcium excretion and, according to some reports, somewhat lower levels of serum ionized calcium. The mean renal calcium clearance is somewhat higher, but the difference from controls did not reach statistical

significance. These data indicate an abnormal handling of a calcium load by patients with EH and raise the possibility that such abnormality may not be due simply to a renal defect but perhaps to an altered calcium distribution among different compartments in the body.
Altered regulation of serum calcium level was proposed to be associated with arterial hypertension and to be dependent on a renal Ca leak or altered Ca binding to plasma proteins and cell membrane described in human and experimental hypertension. Hypertensive patients have an altered regulation of serum Ca concentrations, probably due to a different body distribution of Ca, rather than to altered Ca binding to plasma proteins.
It has been reported that changes in salt loading influence parameters of calcium metabolism in hypertensive subjects. It was also reported that response of blood pressure to salt intake is related to salt-induced increase in intracellular calcium and decrease in intracellular magnesium concentrations. Several authors showed that salt-sensitive hypertensive subjects significantly decreased blood pressure after calcium intake which was emphasized by high salt intake.
It has been showed that during high salt intake regimen increase in blood pressure was followed with decrease in serum calcium level, this was explained by the fact that high salt intake stimulates the Ca uptake by cells. They also reported the following characteristics of hypertensive patients with additionally lower blood pressure as a response to Ca intake: salt-sensitive, low serum ionized Ca and plasma renin activity (PRA) values and high parathyroid hormonE (PTH) values and 1,25-(OH)2-D3 values.
A number of abnormalities in the extracellular and intracellular handling of Ca in arterial hypertension, namely an increased urinary Ca excretion, a reduced serum ionized Ca level and an enhanced intracellular free calcium concentration, have previously been reported. The total body Ca clearance, calculated from the area under the curve of the serum Ca concentrations, was enhanced in hypertensive patients (P less than 0.03). Although the renal Ca excretion is higher in hypertension, the renal calcium clearance account for only a minor fraction of the total body clearance, suggesting that the reduced serum Ca levels achieved by the hypertensive patients are not explained by the renal Ca leak. The enhanced total body Ca clearance found in hypertensive subjects is therefore due to an increased tissue Ca uptake. This finding provides indirect evidence of altered cell Ca handling in hypertension.
Ca metabolism has been investigated in patients with essential hypertension and normal renal function to evaluate the renal calcium handling and the reported increase in renal Ca loss. The results support the hypothesis of primary renal Ca leak in essential hypertension. Enhanced urinary calcium excretion rate may cause compensatory PTH overactivity.
Increased gut Ca absorption or reduced renal tubular Ca reabsorption have been alternatively reported in idiopathic hypercalciuria with kidney calculi. Although renal Ca excretion is higher in hypercalciurics, renal Ca clearance account for only a minor fraction of the total body clearance, suggesting that the reduced serum Ca levels found in the hypercalciurics could not be explained by the renal Ca leak. The enhanced total body Ca clearance found in hypercalciuric subjects is therefore due to an increased tissue Ca uptake. This finding provides indirect evidence of altered cell Ca handling in idiopathic hypercalciura with no difference between the so-called absorptives and renals in terms of the pathophysiologic mechanism.

2.3.1 Testing collagen in urine or blood

Laboratory tests that measure the amount of collagen in urine or blood samples can indicate bone loss. Lab tests may also be used in conjunction with DEXA or other methods of bone

densitometry to diagnose and monitor osteoporosis, such as beta-crosslap, a biochemical bone marker of bone resorption. Biochemical bone markers, such as the bone isoenzyme form of alkaline phosphatase, have been used to assess the bone formation phase of bone turnover in health and disease. Markers of biochemical bone remodeling can be used in assessing and managing osteoporis in conjunction with DEXA.

2.3.2 The active vitamin D

Chronic uremia is characterized by decreased levels of plasma 1,25-dihydroxyvitamin D3(1,25-(OH)2D3), a hormone with immunomodulatory properties, due to decreased renal 1-hydroxylase activity and by decreased renal phosphate excretion. The consequence is an increased synthesis and secretion of parathyroid hormone--secondary hyperparathyroidism--due to the low levels of plasma calcium, low levels of plasma 1,25(OH)2D3 and high levels of phosphate. The association between renal bone disease and chronic renal failure is well described. An association also exists between secondary hyperparathyroidism and increased mortality and cardiovascular calcifications in chronic uremic patients.

Calcium carbonate and calcium acetate were used as phosphate binders. Until recently, the most commonly used active vitamin D drug was either the natural 1,25(OH)2D3, or the 1 alpha-hydroxylated analog, 1alpha(OH)D3 which after 25-hydroxylation in the liver is converted to 1,25(OH)2D3. This increases the intestinal absorption of calcium and improves skeletal abnormalities. The combined treatment with calcium containing phosphate binders and active vitamin D induces an increase in plasma Ca and hypercalcemia became a clinical problem. It was demonstrated a direct suppressive effect of intravenous 1,25(OH)2D3 on plasma PTH.

The use of 1 alpha-hydroxyvitamin D3 (1 alpha(OH)D3) derivatives in a uremic patient is justified only in the treatment of hyperparathyroidism. The following prerequisites have however to be satisfied: a good vitamin D3 repletion should be secured by plasma 25-OH-D3 levels of 20-30 ng/ml, and phosphate retention and the consequent possible hyperphosphatemia should be prevented or corrected by the oral administration of alkaline salts of calcium given before the meals as phosphate binders without inducing hypercalcemia.

In X-linked hypophosphatemia, phosphate wasting results from increased circulating levels of fibroblast growth factor 23 (FGF-23). Administration of calcitonin causes a drop in serum levels of FGF-23. Calcitonin might have the same effect in patients with X-linked hypophosphatemia. Serum levels of 1,25-dihydroxyvitamin D rose similarly in untreated patients with X-linked hypophosphatemia and in controls after a single subcutaneous injection of 200 IU of salmon calcitonin in both groups for 21 hours but diverged thereafter (P=0.008). The rise in serum levels of 1,25-dihydroxyvitamin D is probably due to the direct stimulatory effect of calcitonin on renal 1α-hydroxylase. Both groups had slight and similar changes in serum levels of calcium and PTH. Serum phosphate levels rose after treatment.

Recently, it was reported that osteocytes express the calcitonin receptor and respond to calcitonin with an increase in sclerostin production. Sclerostin has an inhibitory effect on the lifetime of the osteoblast. Sclerostin production by osteocytes is inhibited by PTH.

2.3.3 The PTH, serum Ca, insulin and vitamin D

PTH is the most important endocrine regulator of Ca and phosphorus concentration in extracellular fluid. It enhances the release of Ca from the large reservoir contained in the bones. Bone resorption is the normal destruction of bone by osteoclasts, which are indirectly

stimulated by PTH. Stimulation is indirect since osteoclasts do not have a receptor for PTH; rather, PTH binds to osteoblasts, the cells responsible for creating bone. In the kidney it enhances active reabsorption of Ca and magnesium from distal tubules and the thick ascending limb. It enhances the absorption of calcium in the intestine by increasing the production of activated vitamin D.

Patients with primary hyperparathyroidism have impaired glucose tolerance more often than do controls, and parathyroid resection sometimes improves this derangement. However, it is unclear whether serum Ca or PTH is more strongly related to impaired glucose metabolism in subjects without primary hyperparathyroidism. Multiple regression analyses showed that the significant and positive correlations between serum Ca vs fasting plasma glucose and homeostasis model assessment insulin resistance in men still remained after adjustment for intact PTH as well as age, body weight, height, creatinine, albumin, phosphate, bone metabolic markers, and estradiol ($P < .05$). Serum Ca level is positively associated with impaired glucose metabolism, independent of PTH or bone metabolism, in men with type 2 DM.

In the relationship between biochemical parameters, parathyroid adenoma volume, and bone mineral density with respect to intact parathyroid hormone (iPTH) levels in patients with primary hyperparathyroidism, it was found there was no correlation between iPTH, serum calcium levels and total T scores at the femur and lumbar spine. After excluding patients with 25-(OH)D3 insufficiency, there was still no correlation between serum iPTH and calcium levels. Parathyroid adenoma volume, serum iPTH and calcium levels were also not different between patients with and without 25-(OH)D3 insufficiency.

Primary hyperparathyroidism (PHPT) and vitamin D insufficiency are two very frequent conditions. Vitamin D treatment is recommended and may decrease PTH levels in PHPT. However, there is no randomized controlled trial to prove any beneficial effect. For safety reasons, it is recommended to monitor plasma and urinary Ca during treatment. Furthermore, the effect of vitamin D repletion on other outcomes like quality of life, muscle function and central nervous system symptoms should be assessed.

2.3.4 Ghrelin and bone mass density

Serum ghrelin is positively correlated with trabecular BMD in a cohort of elderly healthy Italian women. The fact that trabecular is more metabolically active than cortical bone and the larger number of females might explain this selective association.

Previously undetected contributors to secondary osteoporosis and metabolic bone diseases (SECOB) are frequently found in patients with osteoporosis, but the prevalence in patients at the time they present with a clinical fracture is unknown (Napoli et al. 2011). At presentation with a fracture, 26.5% of patients have previously unknown contributors to SECOB, as monoclonal proteinemia, renal insufficiency grade III or greater, primary and secondary hyperparathyroidism, hyperthyroidism, and hypogonadism in men. Newly diagnosed SECOBs, serum 25-hydroxyvitamin D less than 50 nmol/liter (in 63.9%), and dietary calcium intake less than 1200 mg/d were found at any age, in both sexes, after any fracture (except SECOB in men with finger and toe fractures) and at any level of bone mineral density, which are treatable or need follow-up, and more than 90% of patients have an inadequate vitamin D status and/or calcium intake. Systematic screening of patients with a recent fracture identifies those in whom potentially reversible contributors to SECOB and calcium and vitamin D deficiency are present (Bours et al., 2011).

2.3.5 Calcitonin and PTH

Calcitonin is a 32-amino acid linear polypeptide hormone that is produced in humans primarily by the parafollicular cells (also known as C-cells) of the thyroid, and in many other animals in the ultimobranchial body. It acts to reduce blood Ca, opposing the effects of PTH.
The hormone participates in calcium Ca and phosphorus metabolism. In many ways, calcitonin counteracts PTH.

2.3.6 Environmental contaminants

Polybrominated diphenyl ethers (PBDEs) are flame retardants that have been widely used in manufacturing. They are major household and environmental contaminants that bioaccumulate. Humans are exposed primarily through dust inhalation and dietary ingestion of animal products. PBDEs increase rodent circulating T3 and T4 concentrations and gonadal osteopontin mRNA, and activate the osteopontin gene promoter. These changes may have clinical implications as others have shown associations between human exposure to PBDEs and subclinical hyperthyroidism (Blake et al., 2011).

2.3.7 Vitamin K_2 (menaquinone)

Vitamin K_2 (menaquinone), is itself a category of vitamin K that includes many types of vitamin K_2. The two subtypes of vitamin K_2 that have been most studied are menaquinone-4 (MK4) and menaquinone-7 (MK7). MK4 is produced via conversion of vitamin K_1 in the body, in the testes, pancreas and arterial walls. Studies demonstrate that the conversion of vitamin K1 to MK4, is not dependent on gut bacteria .
In contrast to MK4, MK7 is not produced by humans but is converted from phylloquinone in the intestines by gut bacteria. However, bacteria-derived menaquinones appear to contribute minimally to overall vitamin K status. MK4 has been approved for the prevention and treatment of osteoporosis, and it has been shown to decrease fractures up to 87%. MK4 has also been shown to prevent bone loss and/or fractures caused by corticosteroids, anorexia nervosa, cirrhosis of the liver and postmenopausal osteoporosis. MK7 has never been shown in any clinical trials to reduce fractures and is not approved by any government for the prevention or treatment of any disease. MK7 has been approved in the purpose of increasing bone mineral density.

3. Overture

BMD is only bone mineral density, risk factors for osteoporosis are only risk factors and the mixing of both parameters does not make quite more sense. It is not better than each one separately. BMD is a subrogate parameter for diagnosing bone strength that is good but it is not enough, because with a suitable BMD caused by sodium fluoride bone fragility is increased and some individuals with decreased BMD undergo quantitatively and objectively bone fractures and another different person does not suffer this bone condition. Bone risk factors are good for diagnosis but they do not mean necessarily one disease and nor are they sufficient to osteoporosis. With and without them there are persons with and without suitable bone strength and with and without fractures. It is important to understand that bone is not a hard and lifeless structure; it is, in fact, a complex, living tissue.
The confounding effect of differences in bone size is due to the missing depth value in the calculation of BMD. It should be noted that despite DXA technology' s problems in estimating volume, it is still a fairly accurate measure of BMD.

Methods to correct for this shortcoming include the calculation of a volume which is approximated from the projected area measure by DXA. DXA BMD results adjusted in this manner are referred to as the bone mineral apparent density (BMAD) and are a ratio of the bone mineral content versus a cuboidal estimation of the volume of bone. As aBMD, BMAD results do not accurately represent true bone mineral density, since they use approximations of the bone's volume.

It is important to get repeated BMD measurements done on the same machine each time, or at least a machine from the same manufacturer. Error between machines, or trying to convert measurements from one manufacturer's standard to another can introduce errors large enough to wipe out the sensitivity of the measurements.

It is possible to use a scaling system for pixels which has a one to one correspondence to the concentration of what you are studying. Sample concentrations can be determined using optical, electronic, and most importantly for our purposes, a computer based imaging technique. Densitometric science was described originally by Bouguer and Lambert who described loss of radiation (or light) in passing through a medium. Later, Beer found that the radiation loss in a media was a function of the substance's molarity or concentration.

According to Beer's law, concentration is proportional to optical density (OD). The logarithmic optical density scale, and net integral of density values for an object in an image is the proper measure for use in quantitation. By Beer's law, the density of a point is the log ratio of incident light upon it and transmitted light through it.

OD = Log10(Io / I)

When dealing with noisy data if there is a region of interest (ROI) or image area that is calibrated, such as is done during concentration calibrations, which method for calculation of a the calibrated mean is preferable?

1. Adding up a calibrated value for each pixel in terms of the calibrated unit, then finding the average calibrated unit value and calling this the calibrated mean.
   Calibrated mean = (sum(cvalue(P[i,j])) / N where cvalue(P[i,j]) is the calibrated value for each pixel in your ROI, and N is the total number of pixels in the ROI
2. Adding up all the pixel values in pixel intensity units, finding the mean pixel intensity value, finally finding the one calibrated value for the mean pixel intensity and calling this the calibrated mean.

Calibrated mean = cvalue(sum(P[i,j]) / N) where P[i,j] is each pixel intensity in the ROI, and N is the total number of pixels in the ROI. In an ideal world, it would not make any difference. Both methods would yield the same value. However in the real world, measurement and other types of error enter in, and we should think of the problem in a statistical context. If the errors (i.e. the standard deviation) are small, the method used does not matter much. But how small is small? What really matters is the relationship of the standard deviation to the curvature of the calibration curve.

If the calibration curve were truly linear, the order of operations would not matter (a property of linear functions). However, in the current context, the calibration curve is always nonlinear, at least in some regions.

The key question then becomes which of the two methods is appropriate on the data? The answer is: it depends. Some cases are clear cut others are in-between. It is safe to assume that, if there is a fairly uniform grey level region of interest, where the only variation is caused by the noise of the imaging process (all noises), method two produces a better estimation of the mean. In cases where the region contains two highly differing density

regions included in one ROI (the variation is not caused by noise or the imaging process), then the method one produces a better estimation of mean.
The error of method one is directly proportional to the noise of the system used and becomes highest when data is measured nearest the asymptote of the curve fit used to calibrate the data. Most data unfortunately have some natural density variation and some variation caused by the imaging process (noise).
We are facing a big concern: Osteoporosis is a major public health threat. How can we treat it if we have not the adequate diagnosis tool?.
An expert technical assessment of the many factors that influence the risk of osteoporotic fracture in postmenopausal women need to be considered when planning the most effective public health interventions. In view of growing awareness of the need to prevent and treat postmenopausal osteoporosis, it is good to resolve several controversies concerning the usefulness of screening programmes, the appropriate target populations, the most effective methods for predicting fracture risk, techniques for assessment, and the comparative effectiveness of currently available preventive and therapeutic interventions. There are advantages and limitations of the methods for predicting future fracture risk: assessment of bone mass, assessment of bone loss, and clinical assessment of risk factors. It is needed information on non-invasive physical techniques for bone mass assessment. The aims and design of screening programmes are not clear.
By reason of the two-dimensional nature of DXA, assumptions must be made regarding the tridimensional nature of the bones involving a great deal to cope with. Therefore it is deduced, that this method seems to be very sensitive to error, and it is necessary to know how to deal with these errors, especially with the systematic errors introduced by using a parameterized model. Even though a high concordance between the densitometers was observed on a single measurement occasion, a significant discordance in longitudinal changes in BMD was observed.
Bone strength is comprised of many components, which include architecture, geometry, cortical porosity, and tissue mineralization density. These components are contained within the measurement of BMD but cannot be individually distinguished.
Bone strength is comprised of many components including, but not limited to bone architecture, geometry, cortical porosity and tissue mineralization density.
The exceptional mechanical properties of bones are not only the result of the amount and type of the micro-constituents, but also of their morphological organization at the different lower scales.
Mechanical properties of bone are determined not only by BMD, but also by tissue trabecular structure and organic composition. Direct measurement of these components of bone strength may result in improved fracture risk prediction or therapeutic monitoring than is currently possible using the surrogate measure of BMD.
In addition to loading in axial compression, long bones are also and, in fact, primarily loaded in bending. In linear coupled bending and extension of an unbalanced bonded repair the tensile forces are exerted on the bending-created convex surface, whereas compressive forces are exerted on the concave surface. This bending increases the stress intensity in the underlying crack and causes adhesive peel stresses and bending of the repair which can, relative to a repair that is restrained against bending, lead to early failure and certain assumptions must be made about the symmetry of the bone in cross-section at the different ROIs, which are not entirely accurate. Additionally, cortical thickness must be assumed to

be uniform about the circumference of the cross-section. Many of these assumptions are necessitated by the 2-dimensional nature of DXA and may be addressed with 3-dimensional imaging.

The geometric parameters are predictive of fracture risk although they do not seem to be better predictors of risk than a conventional measurement of BMD. DXA measured *in vivo* "BMD" methodology shows to be an intrinsically flawed and misleading indicator of bone mineral status and an erroneous gauge of relative fracture risk.

DXA methodology to provide accurate, quantitative, and meaningful *in vivo* (not in cadaver) area bone mineral density ("aBMD") determinations have been proven to be unwarranted and misplaced. The underlying systematic of sizable, inherently unavoidable and uncorrectable inaccuracies in the DXA output values of *in vivo* "BMD" have been shown to be quantitatively consistent with being the root cause of unreliable, misdirected, and misinterpreted aspects of consensual knowledge of bone fragility, osteoporotic diagnostics/prognostics, and remodelling therapies.

So, as said above, BMD is only BMD, risk factors to osteoporosis are only risk factors and mixing of both parameters does not make quite more sense. It is not best than each of them alone. Although spatial information is currently recorded in the form of a DXA image, this information is not utilised clinically. It should be noted that BMD assessment provides an areal density measure, where the cross-sectional scan area is known but not the tissue thickness, providing units of g/cm2.

Precise in vivo measurement of the trabecular bone mechanical properties is very important, being essential a method for quantitatively and objectively assessing bone mass and anisotropy and not only in a qualitative manner and with risks which sometimes are not. The cortical bone properties constitute another system with microsystems, isotropy and anisotropy and variety of cross-section of the long bone. But the mechanical properties of bones are not only the result of the amount and type of the micro-constituents, but also of their morphological organization at the different lower scales. Measurement of BMD has served as a fit surrogate for the measurement of bone strength. DXA is one osteoporosis imaging diagnosis testing. By reason of the two-dimensional nature of DXA, assumptions must be made regarding the tridimensional nature of the bones, dealing with an inference problem from a set of measurements. It is needed to make inference about certain parameters which help to make predictions of a certain fracture risk. The main limitation for a proper inference is that only 2d information is got from detectors, and therefore all 3d information is lost, as it is integrated out due to the nature of detector. It is necessary to be very careful when using models for data inference, because we obviously will never know the underlying truth contained in the data. Therefore, it is tried to regain some information about the third dimension by building a model of the bone, which assumes axial symmetry.

By using a model, to be arranged the parameter of this model in such a way that they best fit the data, so it is only gained information about how good the model can explain the data, but it is not gotten any information of how good this model actually is, and maybe there is a much better model, which we do not know it yet. It is very difficult to make good inference of the bone strength due to the noisy character of the data, and dealing with the errors of the apparatus is crucial for making inference.

Therefore it is deduced, that this method seems to be very sensitive to error, and it is necessary to know how to deal with these errors, especially with the systematic errors introduced by using a parameterized model.

There is concern that the additional 3d information which is gained in this inference process comes entirely from the model, which then would increase the systematic uncertainties about the quantities that are inferred from the data.
And there is an anisotropic problem, and therefore different inferences must be done making measurements from different directions. But we wonder should the anisotropies are really that bad problem.
It should be very easy to test the reliability of this method, by making inferences from datasets taken from different sides, and see to what degree they agree, this would give a simple estimate about some of the systematic errors introduced in the inference method, and how reliable the entire method is. If the reliability is sufficiently high for purposes to study then it would be say there is no use in making a more complicated model. A much deeper investigation of these effects can be carried out in the framework of Bayesian statistics, which is very well suited for problems like this.
But if the reliability is not within the desired range, then of course the only way to tackle this problem is to introduce more complexity to the model to also pick up effects coming from the anisotropies. Which would also means more data might be needed. Treating anisotropies in data inference is in general a very hard business, and a lot of work is going on at the moment to tackle this problems.
In this case we have good chances of attacking this problem, because the anisotropies which might occur are not so nasty, so it might be feasible to build a slightly more general model by allowing elliptic shapes, which introduces two parameters a(x) and b(x) for semi and major axis at each point x along the bone axis, or use other Kernel functions which can describe the shape more precisely.

3.1 The finite element method

The finite element method (FEM), its practical application often known as finite element analysis (FEA), is a numerical technique for finding approximate solutions of partial differential equations (PDE) as well as of integral equations. FEA was first developed in 1943 by R. Courant, who utilized the Ritz method of numerical analysis and minimization of variational calculus to obtain approximate solutions to vibration systems. FEA has been developed to an incredible precision. It consists of a computer model of a material or design that is stressed and analyzed for specific results. It is used in new product design, and existing product refinement. Modifying an existing product or structure is utilized to qualify the product or structure for a new service condition. In case of structural failure, FEA may be used to help determine the design modifications to meet the new condition.
FEA is a widely-used technique for the computer modelling of structures under mechanical loading. A finite element is an individual regular shape thathas a known stiffness so that any applied load will give a predictable corresponding displacement. Elements are joined together at nodes and along edges. Complex designs are created as an assembly of elements to which restraints and loads may be applied. During the computer analysis of the model, a series of simultaneous equations are established that represent the overall stiffness of the structure. The equations are then solved giving the nodal displacements resulting from the applied loads. For the analysis of bone structures, finite element analysis would therefore be dependent upon the density of each element, the arrangement of elements (eg trabecular structure), the composition (eg cortical shell or cancellous) and the external shape (eg length, angle and width of femoral neck).

FEA has previously been applied to computer modelling of several bioengineering situations incorporating bone including cellular remodelling, prosthetic loosening, fracture progression and fracture healing. Studies related to osteoporosis have tended to utilise the full 3D potential of FEA via incorporation of computed tomography data.
FEA predicts the mechanical behaviour (displacement or stress) of a structure under loading rather than the exact yield point (fracture); but since osteoporosis fracture risk assessment requires only a proportional, rather than exact, measure of fracture load, FEA derived stiffness (load / displacement) should have significant clinical potential. FEXI (finite element analysis of x-ray images) provides a thin plate computer simulation of a bone being mechanically tested.
Finite element analysis inherently offers dependence upon the external shape and internal structure of a bone and should, therefore, have the potential to provide a superior prediction of mechanical integrity than simple areal density (BMD). The novel feature of the FEXI approach is that a conventional mechanical compression test is simulated. An important aspect of the technique is that, being based upon conventional 2D DXA images or radiographs, it could be readily utilised into routine clinical practice.
Thus, bone microarchitecture and biomechanical properties in men have been investigated (Vilayphiou et al. 2011). Patient-specific finite element (PSFE) models based on QCT are generally used to predict the biomechanical response of human bones with the future goal to be applied in clinical decision-making (Trabelsi & Yosibash, 2011). The biomechanical mechanisms underlying sex-specific differences in age-related vertebral fracture rates are ill defined. To gain insight into this issue, we used finite element analysis of clinical CT scans of the vertebral bodies (Christiansen et al., 2011).

4. Conclusion

Therefore it is necessary to carry out more research and to open new tracks to have any further reliable tool in the diagnosis of osteoporosis. Precise *in vivo* measurement of the bone mechanical properties is very important, being essential a method for assessing quantitatively and objectively bone mass and anisotropy and not only in a qualitative way and with risks which sometimes are not. Thus, a mathematical, physical and physiological 5-dimensional model must be developed in order to gauge bone properties including geometry(2-dimensional DXA), space, time, motion and stress with some portable-computer-devices that uses the body space of the user as an interface with equipment and programs designed to communicate information from one system of computing devices and programs to anothers. Because the person is not one body died, and is more than one statistic sampling; he is not a 10-year probability of hip fracture; he is alive and not one lifeless inert element; and bones are not quite as strong as one compact material object without life; it is somewhat more flexible and this is useful in bones that are jointed performing its necessary task. Probability is good after the event but not before. It is unknown what is going to happen to one person as the justification is wholly independent of sense experience in *a priori* knowledge. The person to study can be the case who is no concluded from the probability. The probability is not the reality and the patient, to a greater or lesser extent, is not a probability that is to say it is one sophism: a plausible but fallacious argument or deceptive argumentation. This is one poor approach to diagnosis in

osteoporosis. There is not any disease but one ill person and so must be considered irrespective of other philosophies including economic resources. These facts are essential in drawing up any test for diagnosis in osteoporosis.

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8

Approach to the Screening and Diagnosis of Osteoporosis

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1. Introduction

The goal of treatment of osteoporosis is to decrease the risk of fractures in patients with high risk for a first or subsequent fracture. The efficacy of treatment will depend on the efficacy and level of accomplishment of case finding to select patients at risk, the results of additional investigations, the efficacy, tolerance, and safety of medical intervention, and the adherence to treatment during follow-up. Each of these steps is critical in treatment in daily clinical practice. Failure to consider one or other step can result in suboptimal fracture prevention or overtreatment (Geusens, 2009).

On the other hand, measurement of bone mineral density (BMD), assessment of the fracture risk, and making decisions regarding to appropriate therapeutic intervention are the ultimate goal when evaluating patients for osteoporosis (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). Since many fractures among postmenopausal women occur in those with T-scores better than in the osteoporotic range (Siris et al, 2004; Schuit et al, 2004; Cranney et al, 2007), screening of the patients at high risk of fracture and early diagnosis are important.

2. Screening of osteoporosis

The aim of screening is obviously to direct interventions to those most in need, and to avoid treatment of healthy individuals who will never fracture. Bone mass is used conventionally as a proxy of overall bone strength and low bone mass is a major risk factor for osteoporotic fractures. Although BMD measurement is the standard test for the diagnosis of osteoporosis before fracture, ongoing research indicates that BMD measurement alone may not be adequate for detection of individuals at high risk of fracture (Kanis, 1994). Epidemiological studies have shown that a substantial proportion of osteoporotic fractures occur in postmenopausal women who do not meet BMD criteria for osteoporosis defined according to the WHO definition as a T-score of -2.5 or below (Siris et al, 2004; Schuit et al, 2004; Cranney et al, 2007). This suggests that factors other than BMD contribute to a patient's risk of fracture. Central dual-energy x-ray absorptiometry (DXA) is not available everywhere. Furthermore, although BMD measurement is specific, it lacks sensitivity when used alone, so that a number of high-risk patients escape identification (Kanis, 1994). Thus, the potential

impact of extensive population-based screening with BMD in women at the time of menopause on the burden of fractures is less than optimal; screening the general population with BMD would not be cost-effective and is considered inadvisable in many countries (World Health Organization [WHO], 2004). In practice, most guidelines recommend using risk factor assessment tools such as Fracture Risk Assessment Tool FRAX® to help select patients for BMD measurement and/or treatment.

The National Osteoporosis Foundation (NOF), US Preventive Services Task Force (USPSTF), and the American Association of Clinical Endocrinologists (AACE) recommend that BMD testing should be performed to guide treatment decisions, based on the patient's risk profile (National Osteoporosis Foundation [NOF], 2003; US Preventive Services Task Force [USPSTF], 2002; Hodgson et al, 2001). Also, the NOF recommends that all postmenopausal women and men age 50 and older should be evaluated clinically for osteoporosis risk in order to determine the need for BMD measurement and considered the possibility of osteoporosis and fracture risk in men and women, based on the presence of the risk factors and conditions (NOF, 2010).

The National Osteoporosis Guideline Group (NOGG) recommends that patients are identified opportunistically using a case-finding strategy on the finding of a previous fragility fracture or the presence of significant clinical risk factors because, at present, there is no universally accepted policy for population screening in the UK to identify individuals with osteoporosis or those at high risk of fracture (Compston et al, 2009).

3. Diagnosis of osteoporosis

Osteoporosis is diagnosed on the basis of either a low-impact or fragility fracture or a low BMD. A low-impact fracture is one that occurs after a fall from standing height or less; a fragility fracture occurs spontaneously or with no trauma (cough, sneezing, sudden movement) (Mauck & Clarke, 2006).

Until recent years, diagnosis of non-fractured patients was based on the quantitative assessment of BMD, usually by central DXA. In 1994, the World Health Organization (WHO) developed a definition of osteoporosis on the basis of studies of women of various ages (Table 1) (Kanis et al, 1994). The BMD, measured with DXA, results are reported as a density measurement in gm/cm^2, in addition to T- and Z-scores.

Category	Fracture Risk	Action
Normal T-score at -1.0 or above	Below average	Be watchful for clinical triggers
Osteopenia T-score between -1.0 and -2.5	Above average	Consider prevention in peri- or post-MPW Be watchful for clinical triggers Possibly repeat investigations in 2-3 years
Osteoporosis T-score at -2.5 or below	High	Exclude secondary causes Therapeutic intervention indicated in most patients
Severe Osteoporosis T-score at -2.5 or below and already experienced one or more fractures	Established osteoporosis	Exclude secondary causes Therapeutic intervention indicated in most patients

Table 1. Definition of osteoporosis by the WHO

The T-score represent the number of SDs from the mean bone density values in normal gender-matched young adults. T-score is used to make a diagnosis of normal bone density, osteoporosis or osteopenia in postmenopausal women and in men age 50 years and older (Leib et al, 2004). Z-scores represent the number of SDs from the normal mean value for age- and gender-matched control subjects. A Z-score of -2.0 or lower may suggest the presence of a secondary cause of osteoporosis, although no definitive data support this hypothesis. Z-scores are used preferentially to assess bone loss in premenopausal women and men younger than age 50 years. A Z-score of -2.0 or lower is defined as "below the expected range for age"; a Z-score above -2.0 is "within the expected range for age." (Leib et al, 2004).
Originally, the definition of osteoporosis was developed for the estimation of the prevalence of osteoporosis across populations. It was not for the assessment of osteoporosis in individual patient. In other words, diagnostic thresholds differ from intervention thresholds. The fracture risk varies at different ages, even with the same T-score. Other factors that determine intervention thresholds include the presence of clinical risk factors (CRFs), costs and and benefits of treatment.

4. Determination of fracture risk

In the past decade, a great deal of research has taken place to identify factors other than BMD that contribute to fracture risk. The consideration of well-validated CRFs, with or without BMD, is likely to improve fracture risk prediction and the selection of individuals at high risk for treatment. Some of these risk factors act independently of BMD to increase fracture risk whereas others increase fracture risk through their association with reduced BMD (e.g., some of the secondary causes of osteoporosis) (Table 2) (Compston et al, 2009).
Several models have been proposed to stratify osteoporotic fracture risk. These include strategies to identify patients with a high risk of low BMD (e.g., the OST index (Gensens et al, 2002), and the FRAX algorithm (Kanis, et al, 2008a, 2008b)) or with a high absolute risk of fractures based on CRFs, with or without BMD (e.g., FRAX algorithm (Kanis, et al, 2008a, 2008b)), Fracture Risk in Glucocorticoid Users (FIGS) (van Staa et al, 2005), the Garvan algorithm (Nguyen et al, 2008)), and simplified questionnaires.
Among these models, FRAX®, developed by WHO is an algorithm for individualized fracture risk prediction which is depend on population-based cohort from Europe, North America, Asia, and Australia.

4.1 Use of WHO Fracture Risk Assessment Tool (FRAX®)

FRAX® is a clinical tool for case finding for identifying patients at high risk for fractures, for selecting patients to measure BMD, and for treatment decisions. FRAX® should not be considered a gold standard but rather provides an aid to enhance patient assessment. The aim of FRAX® is to provide an assessment tool for the fracture prediction with use of CRFs with or without femoral neck BMD (Kanis et al, 2008a). These CRFs include age, sex, race, height, weight, body mass index (BMI), a history of fragility fracture, a parental history of hip fracture, use of oral glucocorticoid, rheumatoid arthritis, and other secondary causes of osteoporosis, current smoking, and alcohol intake of three or more units daily. These risk factors were identified and validated based on an analysis of 12 prospective studies, yielding a total of 250,000 person-years in 60,000 men and women with more than 5,000 osteoporotic fractures (Kanis, 1994). Because fracture probability also varies markedly among different regions of the

world, FRAX® allows fracture risk to be calculated for countries where the incidences of both fractures and mortality are known (Unnanuntana et al, 2010).
FRAX® has been developed for calculating the 10-year absolute fracture risk in individual patients in primary care settings for a major osteoporotic fracture (in the proximal humerus, the wrist, or the hip or a clinical vertebral fracture) and for a hip fracture calibrated to the fracture and death hazards. The relative risks are difficult to apply in clinical practice since their clinical significance depends on the prevalence of fractures in the general population. As a result, the concept of the absolute risk of fractures has emerged and refers to the individual's risk for fracture over a certain time period, e.g., over the next 5 or 10 years which is the usual duration of the effects of osteoporosis medications during and after use (van Geel et al, 2010).
The FRAX® algorithm is available at www.nof.org and at www.shef.ac.uk/FRAX. FRAX® is intended for postmenopausal women and men age 50 and older who have not been treated for osteoporosis; it is not intended for use in younger adults or children.
NOF starts case finding with age as a criterion (NOF, 2011). Below 65 years, NOF advocates clinical attention for the presence of CRFs (those included in FRAX, with the addition of other risks), and a DXA in the presence of CRFs. In all women older than 65 years, NOF advocates BMD. Treatment is advocated in women with osteoporosis or osteoporotic fracture and in women with osteopenia if FRAX® calculation with BMD indicates a high risk of fracture or when specific high risks (total immobilization and glucocorticoid use) are present.

Age
Sex
Low body mass index (≤19 kg/m^2)
Previous fragility fracture, particularly of the hip, wrist, and vertebrae including morphometric vertebral fracture
Parental history of hip fracture
Current glucocorticoid treatment (any dose, per oral for ≥3 months)
Current smoking
Alcohol intake of 3 or more units daily
Secondary causes of osteoporosis including:
Rheumatoid arthritis
Untreated hypogonadism in men and women
Prolonged immobility
Organ transplantation
Type 1 diabetes
Hyperthyroidism
Gastrointestinal disease
Chronic liver disease
Chronic obstructive pulmonary disease
Falls*

* Not presently accommodated in the FRAX® algorithm

Table 2. Clinical risk factors used for the assessment of fracture probability

The National Osteoporosis Society (NOS) starts case finding with CRFs of FRAX® in all postmenopausal women (Compston et al, 2009). Treatment is advocated in high-risk patients based on CRFs of FRAX® without DXA and in patients with intermediate risk when BMD results integrated in FRAX® indicate a high risk.
It should also be acknowledged that there are many other risk factors for fracture that are not incorporated into assessment algorithms. FRAX® does not include fall-related risk factors and other risk factors for fractures: dose and duration of some risk factors like glucocorticoid use; characteristics of previous fractures (location, number, and severity); vitamin D deficiency; and levels of biochemical markers of bone turnover (van Geel et al, 2010). Moreover, no randomized clinical trials focusing on prevention of fractures in patients who are included based on FRAX® are available (van Geel et al, 2010). Further studies will be needed on the ability to treatment to reduce fracture risk in subjects at high risk for fractures based on FRAX®. Another drawback is that FRAX® is only applicable in treatment naïve patients (Saag, 2009).

5. Clinical investigations

Comprehensive approach to the clinical evaluation of osteoporosis is recommended. A detailed history and physical examination together with BMD assessment and the 10-year estimated fracture probability are utilized to establish the individual patient's risk. The range of tests will depend on the severity of the disease, age at presentation, and the presence or absence of fractures. The aims of patient evaluation are to exclude diseases that mimic osteoporosis (e.g. osteomalacia, myeloma), identify the cause of osteoporosis and contributory factors, assess the risk of subsequent fractures and select the most appropriate form of treatment (Compston et al, 2009).

5.1 History and physical examination

Many metabolic bone diseases are associated with low BMD, therefore a complete and thorough history taking and physical examination are essential to establishing a correct diagnosis of osteoporosis. A complete history should be obtained, with specific attention given to the risk factors, including lifestyle, medical, family, and medication histories (Table 3) (NOF, 2010). Physical examination should include height and weight for BMI and determining any loss of height (historical height loss >4 cm). A thorough physical examination may detect kyphosis, a protruding abdomen, rib-iliac crest distance of less than 2 cm, height loss (prospective height loss >2 cm), acute or chronic back pain and/or tenderness, reduced gait speed or grip strength, and poor visual acuity. Certain other findings, such as nodular thyroid, hepatic enlargement, jaundice, or cushingoid features may reveal secondary causes of osteoporosis (Lane, 2006).
Since the majority of osteoporosis-related fractures result from falls, it is also important to evaluate risk factors for falling. The most important of these seem to be a personal history of falling, along with muscle weakness and gait, balance and visual deficits (Anonymous, 2001). All elderly should be asked annually about the occurrence of falls. Any patient who reports a single fall should undergo basic evaluation of gait/balance (e.g., "Get Up and Go test")(Anonymous, 2001). Items that should be included as a part of a fall risk assessment are summarized in Table 4 (NOF, 2010).

Lifestyle factors		
Low calcium intake	Vitamin D insufficiency	Excessive vitamin A
High caffeine intake	High salt intake	Aluminum (in antacid)
Alcohol (≥3 units/day)	Inadequate physical activity	Immobilization
Smoking	Falling	Thinness
Genetic factors		
Cystic fibrosis	Homocysteinuria	Osteogenesis imperfecta
Ehlers-Danlos	Hypophosphatasia	Parental history of hip fracture
Gaucher's disease	Idiopathic hypercalciuria	Porphyria
Glycogen storage disease	Marfan syndrome	Riley-Day syndrome
Hemochromatosis	Menkes steely hair syndrome	
Hypogonadal states		
Androgen insensitivity	Hyperprolactinemia	Turner's & Klinefelter's syndromes
Anorexia nervosa, bulimia	Panhypopituitarism	Athletic amenorrhea
Premature ovarian failure		
Endocrine disorders		
Adrenal insufficiency	Diabetes mellitus	Thyrotoxicosis
Cushing's syndrome	Hyperparathyroidism	
Gastrointestinal disorders		
Celiac disease	Inflammatory bowel disease	Primary biliary cirrhosis
Gastric bypass	Malabsorption	GI surgery
Pancreatic disease		
Hematologic disorders		
Hemophilia	Multiple myeloma	Systemic mastocytosis
Leukemia, lymphoma	Sickle cell disease	Thalassemia
Rheumatic and autoimmune diseases		
Ankylosing spondylitis	Lupus	Rheumatoid arthritis
Miscellaneous conditions and diseases		
Alcoholism	Emphysema	Muscular dystrophy
Amyloidosis	End stage renal disease	Parenteral nutrition
Chronic metabolic acidosis	Epilepsy	Post-transplant bone loss
Congestive heart failure	Idiopathic scoliosis	Prior fracture as an adult
Depression	Multiple sclerosis	Sarcoidosis
Medications		
Anticoagulants (heparin)	Chemotherapeutic drugs	GnRH agonists
Anticonvulsants	Cyclosporin A, tacolimus	Lithium
Aromatase inhibitors	Depo-medroxyprogesterone	Barbiturates
Glucocorticoid	Selective serotonin reuptake inhibitors	Thiazolidinediones
Proton pump inhibitors		

Table 3. Conditions, diseases, and medications that cause or contribute to osteoporosis and fractures

Environmental risk factors
Lack of assistive devices in bathrooms
Loose throw rugs
Low level lighting
Obstacles in the walking path
Slippery outdoor conditions
Medical risk factors
Age
Anxiety and agitation
Arrhythmia
Dehydration
Depression
Female gender
Impaired transfer and mobility
Malnutrition
Medications causing oversedation (narcotic analgesics, anticonvulsants, psychotropics)
Orthostatic hypotension
Poor vision and use of bifocals
Previous fall
Reduced problem solving or mental acuity and diminished cognitive skills
Urgent urinary incontinence
Vitamin D insufficiency (25(OH)D <30 ng/mL (75 nmol/L)
Neuro and musculoskeletal risk factors
Kyphosis
Poor balance
Reduced proprioception
Weak muscles
Other risk factors
Fear of falling

Table 4. Risk factors for falls

5.2 Bone mineral density measurement

Although central DXA of the hip (femoral neck or total hip) is the gold standard for diagnosing osteoporosis, many experts including the International Society for Clinical Densitometry (ISCD), recommend using the lowest central DXA T-score of posteroanterior lumbar spine (L1-L4), femoral neck, or total hip (or the 33% distal radius of the non-dominant forearm, if measured) to make the diagnosis (Leib et al, 2004). DXA measurement of BMD at other sites (including the trochanter, Ward triangle, lateral lumbar spine, other forearm regions, heel, or total body) or with other technologies (calcaneal ultrasonography, peripheral DXA, quantitative computed tomography, single- or dual-photon radionuclide absorptiometry, or magnetic resonance imaging) are not recommended for use in diagnosing osteoporosis (Leib et al, 2004; Marshall et al, 1996).

As a spine region of interest, posteroanterior L1-L4 for spine BMD measurement and only exclude vertebrae that are affected by local structural change (e.g., degenerative change or compression fracture) or artifact should be used (Baim et al, 2008). However, BMD based diagnostic classification should not be made using a single vertebra. If only one evaluable vertebra remains after excluding other vertebrae, diagnosis should be based on different valid skeletal site.

As a hip region of interest, femoral neck or total proximal femur, whichever is lowest should be used (Baim et al, 2008). Forearm BMD should be measured under the following circumstances: hip and/or spine cannot be measured or interpreted; hyperparathyroidism; and very obese patients (over the weight limit for DXA table) (Baim et al, 2008).
Peripheral DXA (pDXA), quantitative computed tomography (QCT), and quantitative ultrasound densitometry (QUS) are also capable of predicting both site-specific and overall fracture risk (NOF, 2010). When performed according to accepted standards, these densitometry techniques are accurate and highly reproducible (USPSTF, 2002). However, T-scores from these technologies cannot be used according to the WHO diagnostic classification because they are not equivalent to T-scores derived from DXA (NOF, 2010). Moreover, these measurements are less useful in predicting the risk of fractures of the spine and proximal femur than central DXA (Lane, 2006).
The accuracy of QCT of the spine in predicting spinal fracture is comparable to that of DXA but has the advantage of measuring true volumetric or 3-dementional BMD, in contrast to the areal BMD obtained from DXA (Miller, 1999). QCT can distinguish between cortical and trabecular bone and thus is more sensitive to changes in BMD caused by the higher bone turnover rate of trabecular bone (Brunader & Shelton, 2002). It is also precise enough to detect BMD changes over time, and it can be used to follow the disease state or to monitor the response of osteoporosis therapy (Brunader & Shelton, 2002). For this reason, QCT are not the gold standard at the moment, but are also recommended (if applicable) to evaluate osteoporosis.

5.3 Vertebral fracture assessment

Morphometric vertebral fractures are the most frequent fractures in women and men older than 50 years (Sambrook & Cooper, 2006). Independent of BMD, age, and other CRFs, radiographically confirmed vertebral fracture is a strong predictor of future vertebral, non-vertebral, and hip fracture risk (Lems, 2007). The presence of a vertebral fracture increases the relative risk of future vertebral fractures by 4.4-fold and increases the risk of fragility fractures at other skeletal sites as well (Klotzbuecher et al, 2000). The higher the grade (severity) of the existing vertebral fracture, or the more vertebral fractures present (one, two, or three), the greater the risk for future fractures (Gallagher et al, 2005; Black et al, 1999).
Clinical vertebral fractures represent one out of three to four morphometric vertebral fractures (van Helden et al, 2008). Because most morphometric vertebral fractures are not diagnosed until clinically suspected and imaging by x-ray is performed, vertebral fractures are often missed.
Imaging techniques to detect and evaluate vertebral fractures in clinical practice include plain radiography (x-ray), computed tomography (CT), magnetic resonance imaging (MRI) nuclear bone scanning, and vertebral fracture assessment (VFA). There are differences in each of these in terms of imaging resolution, radiation exposure, availability, cost, and patient convenience.
Vertebral Fracture Assessment (VFA) is a new method to evaluate the presence of morphometric vertebral fractures and deformities using central DXA. VFA reliably and accurately identified patients with vertebral fractures that have not been recognized, with greater patient convenience, lower cost, and less radiation than standard x-ray. VFA is indicated when there is a probability that a prevalent vertebral fracture will influence clinical management of the patient (Lewiecki & Laster, 2006). The use of VFA contributes to better define the fracture risk in women with osteopenia and contributes to treatment decisions identifies patients at high risk of fractures in the absence of BMD-based osteoporosis. Indications for VFA according to the ISCD are presented in Table 5 (Baim et al, 2008).

Postmenopausal women with low bone mass (osteopenia) by BMD criteria, plus any one of the following:
Age ≥70 years Historical height loss >4 cm (1.6 inch) Prospective height loss >2 cm (0.8 inch) Self-reported vertebral fracture (not previously documented) Two or more of the following: Age 60-69 years Self-reported prior non-vertebral fracture Historical height loss of 2 to 4 cm Chronic systemic diseases associated with increased risk of vertebral fractures (for example, moderate to severe chronic obstructive pulmonary disorder (COPD) or chronic obstructive airway disease (COAD), seropositive rheumatoid arthritis, Crohn's disease)
Men with low bone mass (osteopenia) by BMD criteria, plus any one of the following:
Age ≥80 years Historical height loss >6 cm (2.4 inch) Prospective height loss >3 cm (1.2 inch) Self-reported vertebral fracture (not previously documented) Two or more of the following: Age 70-79 years Self-reported prior non-vertebral fracture Historical height loss of 3 to 6 cm On pharmacologic androgen deprivation therapy or following orchiectomy Chronic systemic diseases associated with increased risk of vertebral fractures (for example, moderate to severe COPD or COAD, seropositive rheumatoid arthritis, Crohn's disease)
Women or men on chronic glucocorticoid therapy (equivalent to 5 mg or more of prednisone daily for 3 months or longer)
Postmenopausal women or men with osteoporosis by BMD criteria, if documentation of one or more vertebral fractures will alter clinical management

Table 5. Indications for vertebral fracture assessment using x-ray absorptiometry

5.4 Biochemical markers of bone turnover

Bone turnover is the principal factor that controls both the quality and the quantity of bone in adult skeleton and it can be assessed by measuring biochemical markers in blood and urine samples. Bone turnover markers (BTMs) represent the products of bone formation and resorption that are released into the circulation (Table 6).

Resorption markers
Urine N-telopeptide cross-links of type 1 collagen (uNTx) C-telopeptide cross-links of type 1 collagen (uCTx) Pyridinolines, free and total (Pyr) Deoxypyridinolines, free and total (Dpd) Serum N-telopeptide cross-links of type 1 collagen (sNTx) C-telopeptide cross-links of type 1 collagen (sCTx) Cross-linked C-telopeptide of type 1 collagen (ICTP) Tartrate-resistant acid phosphatase (TRAP)
Formation markers
Serum Bone specific alkaline phosphatase (BSAP) Osteocalcin (OC) Amino-terminal propetide of type 1 collagen (P1NP) Carboxy-terminal propetide of type 1 collagen (P1CP)

Table 6. Markers of bone turnover

Quantitative changes in BTMs reflect the dynamic process of bone metabolism. BTMs have been associated with increased osteoporotic fractures independently of BMD in large prospective studies. They also may predict bone loss and, when repeated after 3 to 6 months of treatment with FDA approved antiresorptive drugs, may be predictive of fracture risk reduction. However, BTMs are not a substitute for DXA in women at risk. The value of BTMs in the assessment of fracture risk is likely to be in combination with risk factors, including BMD (Delmas et al, 2000). Generally, their use in the diagnosis of osteoporosis is not recommended (Lash et al, 2009).

There are multiple factors that may cause variations in the levels of BTMs (Table 7). Therefore it is necessary to review certain factors that affect bone marker levels. The main source of variability is pre-analytical; mostly sample conservation and biological variability (Unnanuntana et al, 2010). Pyridinoline crosslinks are light sensitive and degraded under the influence of intense UV irradiation (Body et al, 2009). Osteocalcin concentrations are decreased by freeze-thaw cycles and hemolysis. Assays detecting only intact osteocalcin are particularly affected by in vitro degradation, so it may be advantageous to use assays recognizing both the intact molecule and the large N-terminal fragment (N-MID, 1–43 amino acid), which appear to be more stable, sensitive and reproducible. (Delmas et al, 1985) Some osteocalcin fragments are also released during bone resorption (Delmas et al, 1990). In adults, the main source of undesirable biological variability is the circadian rhythm, with higher values in the early morning hours (peak in 4:00 A.M. and 8:00 A.M.), then a steep decrease in the morning, to attain a nadir at the end of the afternoon (through in 1:00 P.M. and 11:00 P.M.) (Seibel et al, 2005). Most BTMs follow the same pattern, with the exception of alkaline phosphatase because of its longer half-life. Practically, it implies that the measurement of BTMs must be performed in the same lab using standard procedures; samples should be taken while fasting and always at the same time of day. For the urinary BTMs, it is best to obtain either a 24-hour urine collection or morning second voided urine sample. Creatinine excretion also contributes to the overall variability in the levels of urinary BTMs (Unnanuntana et al, 2010).

Biological factors	Analytical factors
Uncontrollable	Technical variability
Age	Sample conservation and processing
Sex	
Growth	
Menopausal status	
Immobilization	
Recent fracture	
Compromised renal and/or hepatic function	
Medical conditions (diabetes, thyroid disease, etc.)	
Medications (anticonvulsants, GnRH agonists, glucocorticoids, etc.)	
Controllable	
Circadian rhythm	
Menstrual cycle	
Exercise	
Food intake	
Seasonal variation	
Sample handling	

Table 7. Factors affecting levels of bone turnover markers

5.5 Laboratory tests

Among men, 30% to 60% of osteoporosis cases are associated with secondary cause. Among perimenopausal women, more than 50% of cases are associated with secondary causes (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy, 2001). In patients referred for DXA in the clinical context of an osteoporosis clinic, contributors to secondary osteoporosis were already documented in one out of three postmenopausal women, previously undiagnosed contributors were found in an additional 30% of women (Tannenbaum et al, 2002).

General consensus exists among experts that a minimum screening laboratory tests should be considered for all patients who are diagnosed as having osteoporosis prior to treatment. Many experts have also suggested that patients who have osteoporosis and a Z-score of less than -2.0 should have more extensive laboratory tests for secondary cause of osteoporosis. A diagnosis of osteoporosis in men should also prompt a through work-up for secondary causes regardless of their Z-score (Mauck & Clarke, 2006).

The range of laboratory tests will depend on the severity of the disease, age at presentation, and the presence or absence of fractures. In patients with BMD-based osteoporosis or presenting with a clinical fracture or both, diagnostic evaluation is necessary and should include blood cell count, sedimentation rate or C-reactive protein, serum calcium, phosphate, alkaline phosphatase, liver transaminase, albumin, creatinine, thyroid stimulating hormone (TSH) and 25(OH)D_3. According to the clinical features and suspicion, other measurements such as parathyroid hormone (PTH), protein immunolelectrophoresis and urinary Bence-Jones proteins, serum testosterone, sex-hormone binding globulin (SHBG), follicle stimulating hormone (FSH), and luteinizing hormone (LH) in men, serum prolactin, 24-hour urinary cortisol/dexamethasone suppression test, endomysial and/or tissue transglutaminase antibodies, 24-hour urinary calcium and creatinine looking for secondary causes are indicated (Compston et al, 2009). If a specific secondary cause of osteoporosis is suspected on the basis of the history and physical examination findings, further direct testing is indicated.

6. Conclusion

Many factors are associated with osteoporosis and fracture, including low peak bone mass, hormonal factors, the use of certain medications, cigarette smoking, low physical activity, low calcium and vitamin D intake, race, small body size, and a personal or family history of fracture. All these factors should be taken into account when assessing the risk of fracture to determine which patients require further assessment and/or treatment. Clinical guidelines help guide practice but should not replace clinical judgment and patient preferences. The final decision about screening, assessment, and/or treatment is ultimately at the discretion of the physician and the patient.

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9

Early Detection Techniques for Osteoporosis

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1. Introduction

Osteoporosis (OP) is a serious disease and its early diagnosis is very important at the right time. These days, there are some conventional techniques of the diagnosis of this disease but these techniques have their limitations and reliable information is not obtained at the initial stage of the disease. Therefore, a new technique for the detection of OP at an early stage is required to be developed. In the present chapter, a new technique, based on Micro Electro Mechanical System (MEMS) technology, will be discussed to overcome the limitations of earlier techniques.

In the present chapter, main emphasis is placed on the early detection of OP. New types of OP detection techniques, based on the biomechanical, optical and electrochemical principles, will be explained and compared to achieve an improved detection methodology for OP. A new amperometric immunosensor using gold nanoparticles and a novel microfluidics BioMEMS chip as a point of care testing (POCT) technique will be introduced for design, fabrication and characterization.

The chapter covers the study which has been mainly divided into three parts: basic measurements on physical, biomechanical, optical and chemical properties of normal and OP bones and serum; and design and development of amperometric immunosensor and BioMEMS chip. (Ahn C.H et al., 2004, Arnaud C.D at al., 1996 Atkinson, P.J. 1964, Auroux, P.A et al., 2002, Bakker, E., 2006, Berthonnaud L, F., 2002, Bianchi, 2005, Ban C, 2004, Bal S.K, 2002)

An overview of OP research trends with the objectives of the research, and scope and significance of the study, is also presented. Causes and diagnostic techniques for OP will be reviewed in the beginning of the chapter.

2. OP detection & early detection of osteoporosis (EDO)

Since OP is most common among elder people, the overall costs to maintain the healthy body will most likely escalate in the near future. Hence to reduce the sufferings, the best solution is early detection (Bianchi, M.L., 2000, Blair, 2000). The early detection of disease states results in improved treatment outcomes, possibility of living longer a healthy life (Arnaud et al., 1996; Singh, 2006).

Early detection is possible by biomarkers or the "intervening phenotypes" in the biofluids like saliva, serum and urine which can be (a) surrogate measures of any malignancy in the bone;

(b) identifiers of inherited variations associated with disease susceptibility; or (c) "pre-disease" lesions that are highly predictive of subsequent disease(Becerra-Rojas & Jupari, 2001).
Diagnosis of OP at the right time can save from compressive treatments and immobility. Generally, in medical practice, identification of disease is based on recognition of symptoms and also testing specific features to confirm the presence of a particular disease. But for OP, it is hard to predict the disease as it silently creeps into the body with minimal symptoms of back pain, toothache and some hunch-over, etc. These initial symptoms point to the old age also and hence the unaware patient realizes this only after having a fracture in the bone. The unaware human when realizes about the disease due to some bone-fractures. In the hospital, anteroposterior and lateral X-rays of the special bone site are obtained to assess the presence of fractures, using the BMD measurements. Finally, they realize the presence of OP then the treatment becomes unaffordable and the patient becomes bed-ridden as shown in Fig. 2. Hence early detection of OP (EDO) is extremely necessary (see Table 1) with new devices like POCT (point of care technology) device using BioMEMS technology. Micro-fluidic chips are also playing key role to deliver new devices for better health care. The sensitivity and specificity of the POCT device would give earliest possible detection. Several promising directions for detection of bonemarker for OP have been interrogated (Arnaud, 1996; Singh, 2006, Singh, 2007, Singh, 2008, Singh, 2009).
Various researchers have studied OP disease for biological, chemically, physiological and engineering aspects in the past, by using different types of measurement techniques and methodologies (Arnaud, 1996; Korkia, 2002; Raiz, 1997; Singh, 2006). However, newer and newer techniques with new advances in technology, like micro/ nano technology, are being developed.

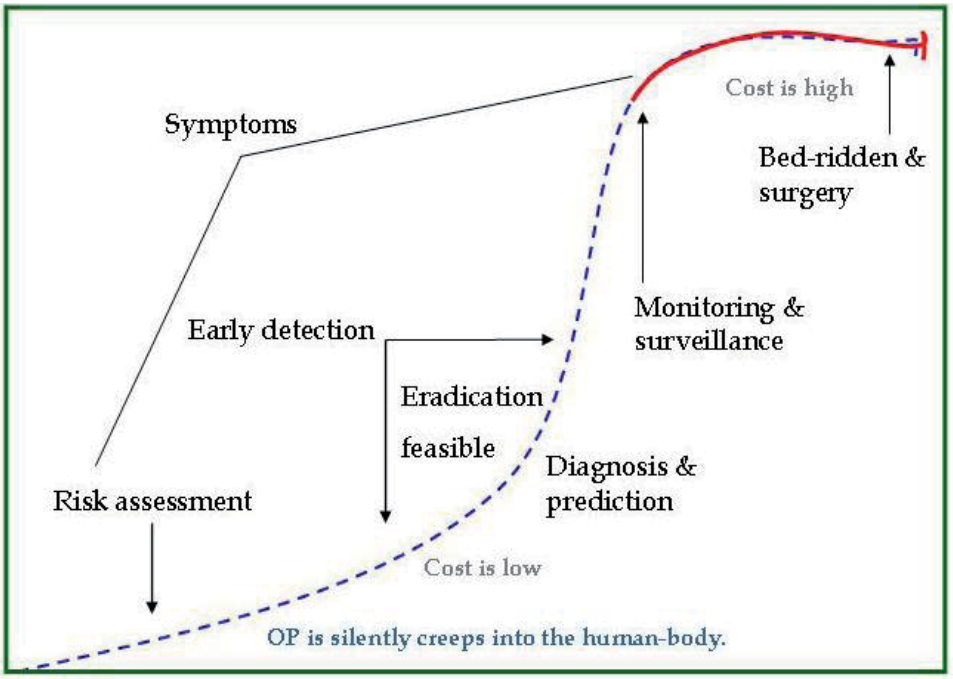

Fig. 1. Early Detection of OP (EDO)

S.No	Specification	Consequences
1	Early detection	Screening for large number of population
2	Minimal invasive	Uses only micro amounts of biofluids
3	Site-specific	Bone turnover specific bonemarkers
4	Diagnostics levels	OP-specific
5	User-friendly	Self testing is possible, easy to use
6	Simple, low cost	Portable, home care

Table 1. Importance of EDO(Singh, 2006)

3. Technical terminologies

3.1 Osteoproteogerin

Osteoprotegerin, also known as osteoclastogenesis inhibitory factor (OCIF), is a cytokine and a member of the tumor necrosis factor (TNF) receptor superfamily. It is a basic glycoprotein comprising 401 amino acid residues arranged into 7 structural domains. It is found as either a 60 kDa monomer or 120 kDa dimer linked by disulfide bonds.

3.2 Biological MEMS (BioMEMS)

It is a combination of a Micro-electromechanical system (MEMS) with the biological systems, like protein, DNA or cell. It includes micro- and nanosystems for genomics, proteomics, and drug delivery analysis; molecular assembly, tissue engineering, biosensor development, and nanoscale imaging (Singh et al., 2007, 2009, Vijayendran et al., 2003).

3.3 Microfluidics

Microfluidics is a multidisciplinary field comprising physics, chemistry, engineering and biotechnology that studies the behavior of fluids at the microscale and mesoscale, that is, fluids at volumes thousands of times smaller than a common droplet. It also concerns the design of systems in which such small volumes of fluids are used (Singh, 2007; Vijayendran et al., 2003).

3.4 Electrochemical sensor

The biosensor in which a biological process is harnessed to an electrical sensor system, such as an enzyme electrode. Other types couple a biological event to an electrical one via a range of mechanisms, such as those based on oxygen and pH (Heineman, 2001, Singh et al, 2008, 2009).

3.5 Immunosensor

Immunosensor uses the immuno-compounds (antibodies or antigens) as biological receptors configures the so-called immunosensors, which are usually the result of the integration in one device of an immunoassay and a directly associated transducer. The antigen-antibody complex formation can be detected either directly (without using any labeled compound) by certain physical (potential, capacitance, conductivity compound) measurements or indirect approaches in which one immuno-compound is conjugated with an indicator molecule (Bakker and Quin, 2006).

3.6 Spectroscopy

It is a non-invasive analytical technique with an infinitely broad range of applications, especially in medical field. This gives a non-destructive analysis. It helps in identification of

the elements and the elucidation of atomic and molecular structure by measurement of the radiant energy absorbed or emitted by a substance in any of the wavelengths of the electromagnetic spectrum in response to excitation by an external energy source (Chittur, 1998; Clark and Hester. 1996).

3.7 Lab-on-a-chip (LOC)

LOC is to integrate multiple functions on a single chip of only millimeters to a square centimeters in size and that are capable of handling extremely small fluid volumes down less than pico liters, LOC device is a sub-set of MEMS device (Chittur, 1998; Clark and Hester, 1996). The term "Lab-on-a-Chip" was introduced later on when it turned out that μTAS (Micro Total Analysis System) technologies were more widely applicable than only for analysis purposes.
(http://en.wikipedia.org/wiki/Lab-on-a-chip).

4. Classification of OP detection techniques

There are several methods for detection of OP as shown in Fig. 2 (Singh, 2006).

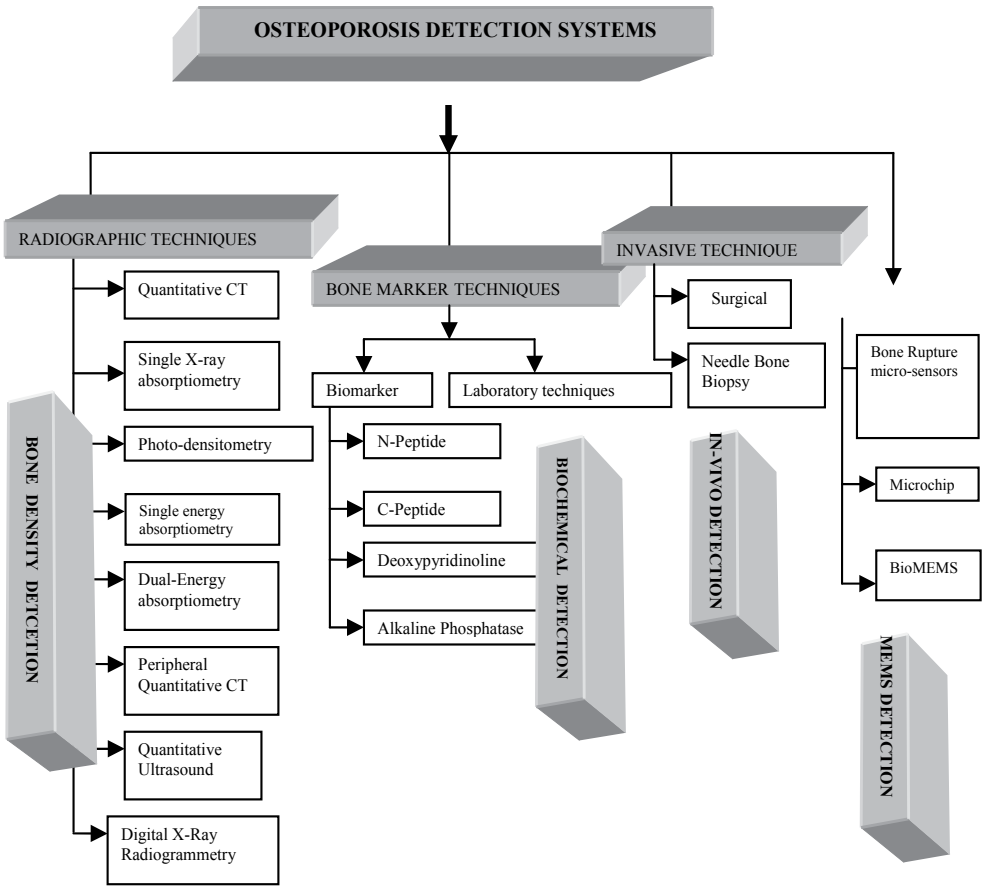

Fig. 2. Classification of Detection Techniques for OP (Singh, 2006)

4.1 Radiographic diagnosis

Radiogrammetry, a technique that has been in use for more than 30 years, relies on the measurement of the cortical thickness of bones in the hand (metacarpals) to estimate bone mass. This technique suffers from relatively poor accuracy and reliability and has largely been supplanted with new techniques(Bianchi, 2000)

The following diagnostic techniques are generally used for the detection of osteoporosis (Arnaud, 1996; Sartoris, 1996, Bianchi, 2005)

Bone Mineral Density Technique (BMD) is an effective approach for detection of osteoporosis. A decrease in the amount of bone, resulting in thin, weakened bones that are susceptible to fractures. Several techniques are available for BMD testing. For example; dual-energy X-RAY absorptiometry (DXA) remains the standard for testing the BMD. Dual energy absorptiometry measures the bone density within a given area of bone (g/cm^2). This technique offers the advantages of higher precision, minimal ionizing radiation exposure, rapid scanning time and the ability to access cortical and trabecular bone mass at appendicular and axial sites. Limitation, include equipment expense, the need for certified X-Ray technician and non-portability. In addition, DXA scans of the spine may show a false increase in spinal BMD in patients with osteophyes, aortic calcifications and degenerative arthritic changes.

The conception of osteoporosis relates bone health to bone strength, rather than mass. A bone's health implies that it should have enough strength to keep voluntary loads from causing spontaneous fractures. Thus, the diagnosis of osteoporosis would be a biomechanical matter concerning both bone strength and muscle strength (Martin et al., 1998). This supposes two kinds of problems, namely: (a) to properly assess bone material properties, structural design and strength; and (b) to correlate the respective indicators with suitable indicators of muscle strength. As a standard densitometry is unsuitable to assess muscle strength or bone strength, it should use of other, preferably cross-sectional analyses of bone structure as those provided by quantitative computed tomography (QCT), peripheral quantitative computed tomography (pQCT), MRI, or similar procedures. The value of pQCT lies in the ability of the software to account for all the `mass', material and architectural factors in whose-bone strength and to provide data on muscle cross-sections (Burr, 1997, Boyle et al, 2003, Singh et al, 2006).

4.2 Quantitative CT

This provides the evaluation of trabecular bone density of the lumbar spine based on bone volume. Quantitative computed tomography (QCT) may be less practical than DXA because of the lower precision, higher cost and increased radiation exposure (Singh et al, 2006).

4.3 Peripheral bone densitometry

These devices used for many devices single-energy X-ray absorptiometry, peripheral DXA and peripheral CT. these device have the advantages of less expense, portable equipment, reasonable precision, and low radiation exposure. The use of quantitative ultrasonography for screening of osteoporosis and assessing fracture risk has increased. Using the speed of sound and broadband ultrasonic attenuation measurements, ultrasonic densitometry provides on bone elasticity and structure in peripheral sites. Advantages of this method, is low cost and lack of ionization radiation (Singh et al, 1997, 1998, 1999, 2006).

4.4 Single-energy absorptiometry

Single-energy absorptiometry measures bone mineral at peripheral sites such as the wrist and heel. Single photon absorptiometry (SPA) used a radioactive energy source, usually iodine125 to estimate the amount of bone mineral at peripheral measurement sites. In recent years, Single-energy X-ray absorptiometry (SXA) has supplanted SPA for measurements of the peripheral skeleton (heel and wrist) because of its better reproducibility and ease of use. SXA avoids the necessity of obtaining and disposing of radioactive energy sources. It requires immersion of the part in water bath and hence can measure bone mass in peripheral bones like bones of forearm and legs (Singh et al, 2006, Blair et al, 2003).

4.5 Dual-energy absorptiometry

Bone density tests are painless, non-invasive and safe. Dual-energy absorptiometry was developed to measure bone in parts of the skeleton (lumbar spine, hip, and total body) that could not be measured with single-energy devices. Currently dual-energy X-ray absorptiometry (DXA) is the most widely used technique for measuring bone at these sites. DXA devices also are capable of measuring bone at the heel and wrist with high accuracy and precision, with very low exposure to radiation (Singh et al, 2006).

4.6 Peripheral quantitative CT (pQCT)

Quantitative computed tomography (QCT) measures the density of vertebral trabecular bone, the spongy bone in the center of the vertebra. pQCT devices are QCT instruments that have been adapted for measurements at peripheral sites such as the wrist (Singh et al, 2006).

4.7 Quantitative ultrasound (QUS)

Quantitative ultrasound devices measure bone at several skeletal sites, including the heel, hand, finger, and lower leg. The heel measurement it is composed of primarily trabecular bone, similar to the composition of the spine. Ultrasound devices based on the changes in the speed of sound (SOS), as well as specific changes in sound waves (broadband attenuation or BUA) as they pass through bone. QUS measurements provide information on fracture risk by providing an indication of bone density and possibly also information on the quality of the bone. Ultrasound devices do not expose the patient to ionizing radiation. Ultrasound devices do not expose the patient to ionizing radiation (Singh, 2006, Sartris, 1996)

4.8 Digital X-ray radiogrammetry (DXR)

Recently, the computer technology has renewed interest in this old technique. The Pronosco X-posure system estimates forearm bone mass from measurements of the cortical width of bones in the hand using computerized digital x-ray radiogrammetry from a single plain radiograph of the hand and wrist. The BMD estimate, referred to as DXR-BMD, is corrected for cortical porosity and striation. The results indicate that this technique is highly reproducible and appears to be at least as good as other peripheral bone assessment techniques in its ability to discriminate among patients with low bone mass at the spine and/or hip and osteoporotic fractures (Sartoris, 1996).

4.9 Photodensitometry

Previously, radiographic absorptiometry (RA) uses standard X-ray images of the hand and distal forearm are taken with a graduated aluminum reference. The radiographic image of

the hand and wrist is captured by a video camera and the levels of grey seen on the hand image are quantified and compared with the grey levels of the reference standard, resulting in an estimate of bone mineral density (BMD). The cortical thickness of the bones can also be measured. Radiographic photo densitometry comprises of comparing the optical density of bone X-ray with standard calibrative, aluminium-step-wedge. Although inexpensive and easily accessible, this method had poor reproducibility. Computer-assisted methods have reduced these errors and several commercial systems have been developed in recent years. Although RA is generally less precise than DEXA, radiographic absorptiometry holds promise as a cost-effective method to screen cases of osteoporosis. Further research is needed to evaluate its effectiveness in predicting fracture and monitoring therapy (Sartoris, 1996, Singh et al, 2006, Blair et al, 2006, Bouxsien and Mary, 2005).

4.10 Double photon absorptiometry

The principle of dual photon absorptiometry (DPA) is the use of a photon beam that has two distinct energy peaks. One energy peak will be more absorbed by soft tissue and the other by bone. The soft tissue component then can be mathematically subtracted and the BMD thus determined (Sartoris, 1996).

4.11 Neutron activation analysis

A limb is bombarded by slow neutron from a generator. This is taken up by the soft tissue to convert it into thermal neutron. This thermal neutron is captured by the nucleus of calcium ion. The nucleus becomes radioactive. Decay of the nuclei emits photon which can be measured by a Geiger counter, giving an idea of bone mass. This is reduced in osteoporosis.
(source: www.iupac.org/publications/pac/1995/pdf/6711x1929.pdf).

4.12 Biochemical techniques

Biomarkers are substances found in an increased amount in the blood, other body fluids, or tissues and which can be used to indicate the presence the presence of osteoporosis. Biomarkers of bone remodeling (formation and breakdown), such as alkaline phosphatase and osteocalcin (serum markers) and pyridinolines and deoxypyridinolines (urinary markers), help in evaluating risk for osteoporosis. The research studies show that biomarkers correlate with changes in indices of bone remodeling and may provide insights into the mechanisms of bone loss which may give a basic detection method. The method may not be precise or accurate but it is quick, early, cheap and non-invasive way of detection. This method gives an indication of the onset of the disease (Sia, 2003).

4.13 Bone markers

There is a need for the development a non-invasive and repeated measurement of bone turnover which demands precision, accuracy and specificity. These kind of independent measurements of bone formation and resorption are done at organ or tissue level. The validated biochemical markers are urine and serum (see Table 2).
The two main biochemical markers for bone formation are serum alkaline phosphatase and serum osteocalcin. Markers for bone resorbtion include urinary calcium and urinary hydroxyproline: Alkaline phosphatase, which reflects osteoclast activity in bone, is measured in serum, but it lacks sensitivity and specificity for osteoporosis, because it can be

elevated or decreased with many diseases. It is increased with aging (see Table 2). Urinary calcium can give some estimate of resorbtion (loss of) bone, but there are many variables that affect this measurement. Urinary hydroxyproline is derived from degradation of collagen, which forms extracellular bone matrix. However, hydroxyproline measurement is not specific for bone, because half of the body's collagen is outside the bony skeleton. It is also influenced by many diseases, as well as diet. Several ELISA kits are developed by Osteomark Company for detection of Osteoporosis (www.osteomark.org).

S.No	Osteoblastic Activity	Osteoclastic Activity
1	S-alkaline phosphatase - Total alkaline phosphatase (S-tAP) - Bone alkaline phosphotase (S-bAP)	U-Hydroxyproline(U-OHPr)
2	S-osteocalcin(S-BGP)	U-collagen crosslinks - Pyridinoline(U-Pyr) - Deoxypryridine(U-D-Pyr)
3	S-carboxyterminal propeptide of human type I collagen(S-PICP)	S-C Terminal pyridine crosslinked telopeptide domain of type I collagen (S-ICTP) S-Tartrate-resistant acid phosphatase(S-TRAP)

Table 2. Urine and serum markers (Sartoris, 1996)

4.14 Laboratory methods

There are several preliminary tests to identify the loss of bone mass. A number of laboratory tests may be performed on blood and urine samples (Singh et al, 2006).
The most common blood tests evaluate:

- blood calcium levels
- blood vitamin D levels
- thyroid function
- parathyroid hormone levels
- estradiol levels to measure estrogen (in women)
- follicle stimulating hormone (FSH) test to establish menopause status
- testosterone levels (in men)
- osteocalcin levels to measure bone formation.

4.15 Needle bone biopsy

Needle bone biopsy is not a very common assessment technique of bone density. This test has limited availability, and is best utilized as a research technique for analysis of treatment regimens for bone diseases. The best clinical use of bone biopsy combines double tetracycline labeling to determine appositional bone growth and rule out osteomalacia. Doses of tetracycline are given weeks apart, and the bone biopsy is embedded in a plastic compound, sliced thinly, and examined under fluorescent light, where the lines of tetracycline (which auto fluoresce) will appear and appositional growth assessed (Singh et al, 2006).

5. Recent novel techniques

Osteoporosis is the disease which creeps into one's body silently without showing a significant symptom. The nature of the disease asymptotic until a gross deformity occurs in one's body. This is can be very serious and deadly for the patient. The time the patient realizes structural support of the body has totally deteriorated. The diagnosis and treatment is sometimes unaffordable for a common man. The patient has the control over prevention of this disease or is reliable, easy and cheap way is available for such deadly disease than proper care can be taken. There are several researches going on in order to achieve some cheap, easy early detection of osteoporosis (Singh et al, 2006). The latest trend is in miniaturizing the device which make it portable, useful for homecare, user-friendly, cheap, Non-invasive and provides a kind early indication for OP. This technique is based on MEMS based technique.

5.1 Bone fracture detection micro-sensor

The method for detection or investigation of osteoporosis is with the help of a micropump (Yung et al, 2004). The micropump has been designed using the electromagnetic principle to actuate the piston in two directions. A closed loop system is used for circulating the fluid with the pumping device. This is kind of pump is useful for blood sampling or drug delivery. Here the oscillating micropump is used to study the mechanosensitivity of bone cell for better investigation of osteoporosis. There is another research which has proposed an implantable, telemetry-based MEMS bone sensor (Singh et al, 2003, 2009) with the capability of determination of bone stress via wireless RF interface. The bone stress is detected using the embedded piezoresistive strain gauges with polysilicon layer and a CMOS chip (Singh, 1997).

Another design of micro-fabricated strain gauge array is used to monitor bone deformation *in vitro* and *in vivo* for detection of osteoporosis. These kinds of microsensor provide a map of distributed strain data over the area of interest on the surfaces of bone to monitor the structural integrity of bone. This type of strain membranes are wireless and implantable embedded in flexible membrane. A simulation experiment was conducted to develop such micro-strain gauge for study of osteoporotic bone (Yang et al, 2004).

A bone sensor has been used for the piezoelectric BioMEMS. An attempt has been made to develop bone-based piezoelectric sensors to detect the stress in bone (Singh, 2003). Another micro-scale sensor for bone surface strain measurement is discussed. This kind of sensor is used for studying the structural effects of osteoporosis. Designs and simulation using ANSYS finite element modeling tool of thin-film metal strain gauge. Metal films for electrical interconnection encapsulated in PDMS have been studied. The PDMS membrane was characterized to facilitate encapsulation designs. The basic fabrication steps like silanization, PDMS preparation, photolithography, PDMS metallization, wire bonding and finally device separation. With experiments were performed for optimizing and characterizing the device like mechanical testing, electrochemical testing and adhesion testing (Yang et al, 2004), and there is a new design which has been discussed here.

Another latest technology is detecting osteoporosis with the study of the brittleness of the bone. The bone mass and bone density play an important role in bone strength. It is

important to measure the brittleness or fragility or the bone mechanics. Certain walking studies are done and it is found that as the heel strikes the ground it creates force pulse, energy that passes up through the body and it is absorbed by bone. The osteoporosis reduces the quality of the bone so by attaching the skin-mounted sensors for measuring the electrical pulses of the muscles which is an active part of the skeletal system. If the person has osteoporosis the energy which passes up to the body is disrupted due to porous nature of the bone (http://www.uc.edu/news/NR.asp?id=3280).

5.2 Microfluidic channels – Detection by biomarkers

The total Alkaline phosphatase (AP) is the mostly widely used bone marker in the clinics and hospitals. AP have physiological substrates which splits the inorganic phosphatase with organic phosphatase, increasing the calcium-phosphatase product and enabling mineralization. AP is essential for normal mineralization of the bone. Bone AP (bAP) constitutes approximately 50% serum AP and the serum has the half-life of 24-48hrs. Though the half-life is relatively large but it may differ on cardiac rhythm, with peak levels in afternoon and night. The exact metabolic pathway is unknown. AP is measured with the help of spectrophotometer using p-nitrophenylphosphate as substrate. The bone and liver AP may be separated by electrophoresis but this method is time consuming and gives semi-quatitaive results. The concentration of bAP concentration may be measured using two antibodies with small differences in affinity toward the isoforms (Singh et al, 2006).

MEMS based detection with alkaline phosphatase has been attempted by Kang and Park (2005). A microfluidic device has been used for enzyme assay. The measurement of enzyme-substrate reaction will to do the substrate consumed. A lab-on-a-chip (LOC) device is developed for controlling the flow containing small volumes of liquids in microchannel which can speeed up and simplifies sample preparation steps in LOC which offers high throughput, low version of traditional research. The microfluidic device is fabricated by the casting process with PDMS. It consists of three parts part I is the injection system, part II is the reaction chamber and part III is the microchannel. This particular microchannel measures the ALP activity using a micro-plate reader. Microfluidic mixing for single enzyme assay was applied and with mathematical prediction the enzymatic (Singh et al, 2006).

5.3 Biochemical based BioMEMS chip

A novel BioMEMS chip, based on gold nanoparticles, for the detection of osteoproteogerin (OPG). This biochip is used to evaluate the bone conditioning which is directly related to the diagnosis and prognosis of the osteoporosis, in an effective manner. The flow visualization of the mixing capabilities are characterized using micro-scale laser-induced fluorescence (LIF). The BioMEMS chip detection is based on competitive immunoassay. The monoclonal OPG antibody (anti-OPG) is immobilized onto the AuNPs deposited conducting polymer, using covalent bonding with a carboxylic acid group. The catalytic reduction is monitored amperometrically at - 0.4 V versus Ag/AgCl. The linear dynamic range is between 2 to 24 ng/ml with the detection limit of 2 ng/ml (Singh et al., 2007). Fig. 3 depicts schematic of the microfluidic chip.

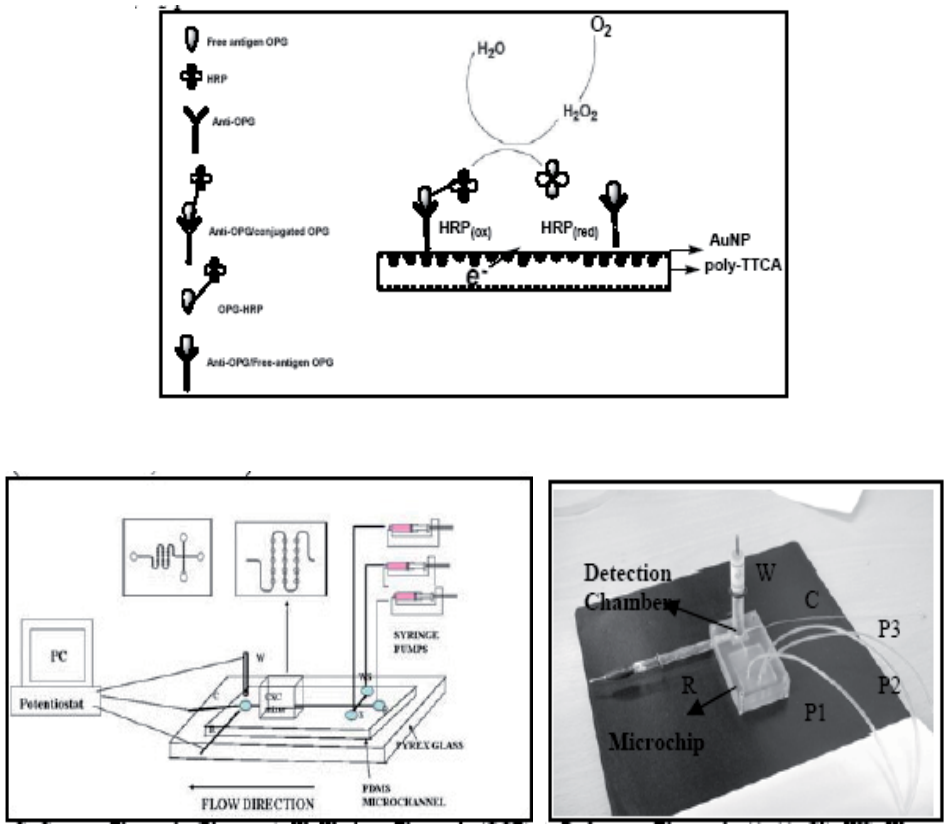

Fig. 3. Microfluidic technique for early detection of OP(Singh et al., 2007 & 2009)

Bone Formation	Bone Resorption
• Osteocalcin (OC) • Bone-specific alkaline phosphatase (BAP) • Amino terminal propeptide of type I collagen (PINP) • Carboxy terminal propeptide of type I collagen (PICP)	• Pyridinoline (Pyr) • Deoxypyridinoline (dPyr) • Amino terminal telopeptide of type I collagen (NTx) • Carboxy terminal telopeptide of type I collagen (CTx)

Table 3. Biochemical Indices (Singh et al., 2006)
(http://www.scielo.br/img/revistas/abem/v50n4/31869t2.gif)

5.4 BioMEMS-based sensors

BioMEMS using electrochemical immunoassay with microfluidic system (Heineman et al, 2001) help in blood sample analysis using the heterogeneous immunoassay. Two concepts of immunoassay are studied in this research. First is based on analogous microcapillary

immunoreactor and other combines the reaction and detection chamber within the area of electromagnet. Both are MEMS based system for alkaline phosphatase study.
Another MEMS microvalve with PDMS diaphragm and two chambers with thermo-pneumatic actuator for integrated blood test system with silicon have been suggested for point of care device. The blood test system can be reduced to reasonable cost with MEMS technology (Singh, 2006). The microvalve with long stroke has been fabricated with two chamber thermo-pneumatic actuator.

5.5 Spectroscopic techniques for early detection of OP

Optical techniques such as Fourier Transform Infrared Spectroscopy (FTIR) and Ultra Violet Visible Spectroscopy (UV-Vis) are employed to find the bone markers with an emphasis on the noninvasive modalities for early detection of osteoporosis. Blood plasma samples procured from two groups, patients and healthy persons were tested. Both of the optical techniques revealed obvious differences in the spectra; between two groups, for example, increase in intensity for OP persons. New peaks were found at 1588, 1456 and 1033 cm^{-1} in FTIR spectra, as shown in Fig. 4. On the other hand, in UV-Visible spectroscopy results, a new peak appeared in the OP patients' spectra at the wavelength of 420 nm, as shown in Fig. 5. These differences in the spectra of the two types of samples, allow rapid and cost-effective discrimination of the potential patients with the optical techniques which were verified by the bone densitometer in the hospitals. The new technique used here is quick, reliable and effective.
A hierarchical algorithm is used to investigate and quantify the mutual relevance between successive clusters in terms of heterogeneity values as shown in the Fig.6.
Fig. 6 represents the classification with the spectra at 1539-1542 cm^{-1}. This gives clear distinction between the patients and healthy groups for the amide II group.

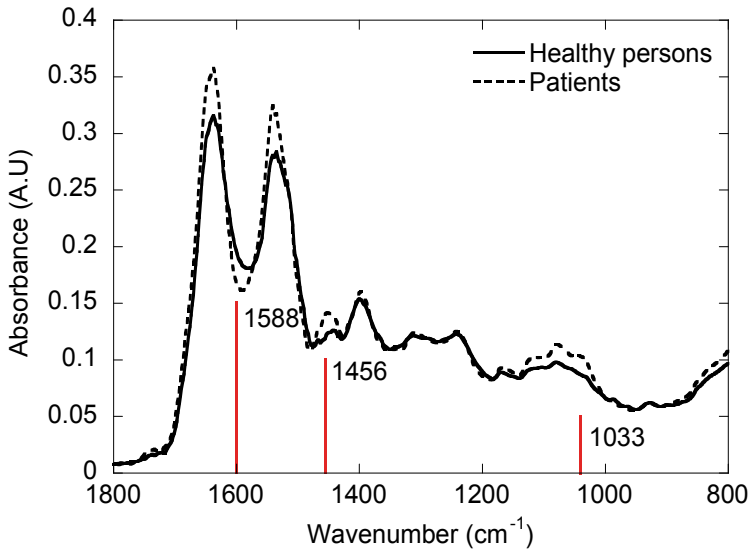

Fig. 4. FTIR results for EDO (Singh et al., 2010)

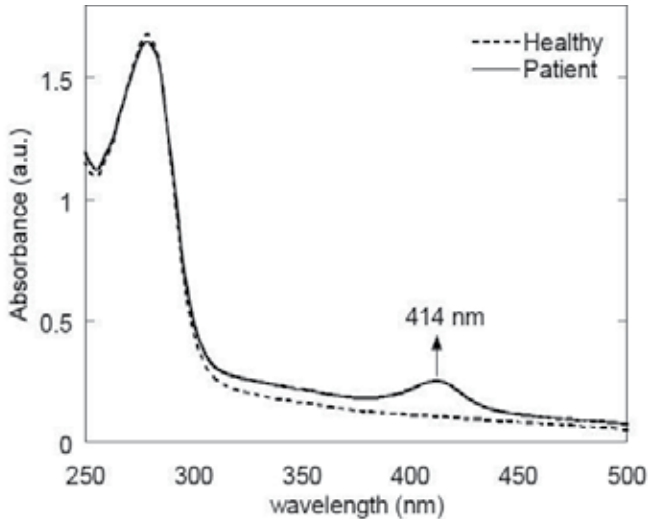

Fig. 5. UV-visible spectroscopy for EDO (Singh et al.,2010)

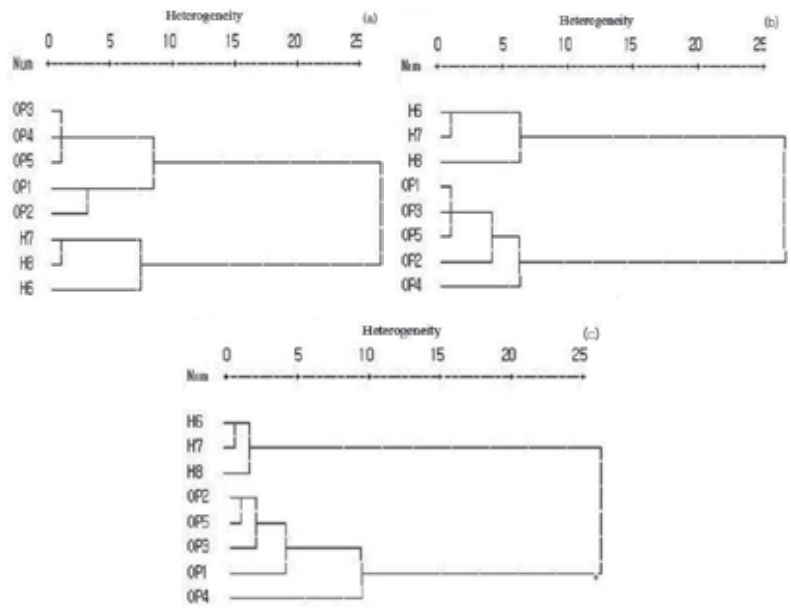

Fig. 6. Cluster analysis(Singh, 2010)

6. Discussions

6.1 Bone density testing

There is several bone density measurement testing techniques which have been discussed with their working principles. The recent developments in the instruments for BMD measurement have also been discussed. But there are several limitations in these devices. The testing with these devices is very expensive, early detection is not easy, these are invasive measurement, errors in magnification may occur, accuracy is not achievable, scan time is high, harmful

radiation may cause problems in the body, home care is not possible and the instruments are not portable. Specially trained persons are required to operate such sophisticated equipments. Though BMD measurement is the most accurate method for detection of Osteoporosis but it is unable to help in early detection and is very expensive. Early detection of such a silent and deadly disease is important for the mankind. Hence there is a need for new, novel, portable, cheap detection systems to be developed for point of care testing.

6.2 Invasive technique

The invasive technique has several risks involved like skin is punctured. There is a slight chance that the needle may cause fracture the bone being sampled or injure one of the nerves, blood vessels, or organs near the biopsy site. If complications occur, another surgery may be needed to treat the problem. After a bone biopsy, there is a slight chance that the bone may become infected, osteomyelitis or not heal properly. In rare instances, the bone from which the biopsy sample was taken may become weak and break, fracture at a later time. This type detection is only good for extreme severe cases.

6.3 Biochemical measurement

There are several bone markers or the biomarkers available for the early detection of the osteoporosis which control the osteoblastic and osteoclastic activity. The biochemical detection is not accurate detection but it gives the indication for onset of osteoporosis detection.

6.4 MEMS-based techniques

The MEMS-based techniques are portable, handheld, easy to use, can be used for home care and point-of-care testing. But the accuracy of the detection may be achieved by using bone mineral density testing (BMD) (Singh et al., 2009, 2010).

7. Conclusions

The radiographic techniques are needed for the accurate detection of osteoporosis as they give precise data for detection of the deadly disease. Generally, the patients are just unaware of the disease as osteoporosis creeps silently within a human body. Osteoporosis is a silent killer and is a very progressive disease. The time the person realizes the detection and treatment becomes unaffordable for patient. This research paper focuses on the urgent need for the development on early, non-invasive, cheap, and handheld POCT device for such a dangerous disease. These characteristics can be achieved by using a micro-size (MEMS/ Nano based techniques). There have been several attempts in this direction as mentioned in the paper above. But this area needs more of research and deep studies.

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10

Sophisticated Imaging Technology in the Assessment of Osteoporosis Risk

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1. Introduction

Osteoporosis is a common disease characterized by low bone mass and microstructural deterioration of bone tissue, with an increased fracture risk. With an aging population, osteoporosis and its related fractures have become an increasingly important health and socioeconomic issue. The aim of osteoporosis screening and treatment is to prevent bone fracture. A fracture occurs when the external force applied to a bone exceeds its strength. The ability of a bone to resist fracture depends on its amount, spatial distribution, and intrinsic properties. Sophisticated bone imaging techniques, as new modalities, improve the potential for non-invasive study of bone anatomy, physiology and pathophysiology. The objective of bone imaging in osteoporosis is to minimize fracture occurrence by identifying the osteoporotic process at an early stage, differentiate distinctive patterns of bone loss, predict fracture risk accurately and monitor treatment response precisely. Non-invasive imaging techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), provide structural information, beyond bone mineral density (BMD). Non-invasive or non-destructive imaging techniques can provide important structural information about the local and systemic skeletal status and about the propensity to fracture. These advanced imaging techniques provide information about bone beyond standard bone mineral densitometry. In this chapter, we will discuss recent progress in bone imaging in a range from the macro- to micro-structures in order to investigate the structural basis of the skeletal fragility underlying osteoporosis.

2. Bone mineral density measurement

In bone fragility assessment, BMD is the main parameter to quantify because of its relationship to bone strength and prediction fracture risk. In the past two decades bone densitometry has been performed with direct methods such as dual X-ray absorptiometry (DXA) and quantitative computed tomography (QCT), which also evaluates bone structural

characteristics. The most commonly used quantitative imaging measure in osteoporosis is the areal BMD assessed by DXA. The assessment of bone macro- and micro-architecture by using more sensitive three-dimensional (3D) methods is important to determine certain aspects of bone structure and quality. Research has especially focused on the assessment of compartmental BMD and bone microstructure, since it has become technologically possible to obtain relatively high resolution volumetric images of bone in vivo.

2.1 Dual X-ray absorptiometry

Bone mineral density (BMD) measurement by dual X-ray absorptiometry (DXA) has been available for clinical use since 1987. It provides a quantitative assessment of mineralized bone mass at the axial and appendicular skeleton in vivo. This technique is currently the most readily available surrogate marker of bone strength and fracture risk. DXA measures the attenuation of photons of two different energies during radiation transmission. Bone mineral content (BMC, g) and areal BMD (g/cm^2) of a region of interest are obtained. As low areal BMD is a strong risk factor for fractures, this technique provides the basis for the World Health Organization (WHO)'s guidelines for diagnosis of osteoporosis. DXA is limited in that it measures only areal BMD two-dimensionally. DXA is also limited in that it does not distinguish cortical and trabecular bone. Furthermore, measurements are subject to artefacts due to degenerative changes such as osteophytes and aortic calcification. Recommendations from the International Society for Clinical Densitometry regarding DXA examination for all ages have been updated. Although DXA is the gold standard for clinical assessment of fracture risk, its shortcomings are increasingly being recognized. Individual fracture risk has recently been standardized using the WHO Fracture Risk Assessment tool (FRAX), which was released in 2009 (Kanis et al., 2009). FRAX combines BMD from DXA with other well-known major risk factors for osteoporosis, such as age, sex and a parental history of hip fracture, to provide a 10-year risk of hip and other major fractures. Although it is not an ideal system, FRAX represents an important initiative in allowing clinicians to individualize fracture risk based on DXA examination and other factors.

2.2 Quantitative computed tomography (QCT)

In quantitative computed tomography (QCT), the X-ray source and detector rotate in synchronised fashion around the subject. Algorithms are used to reconstruct the attenuation data into 3D images. Use of a bone mineral or hydroxyapatite phantom allows calibration of the data, providing a measurement of bone density that is independent of bone size. Compared with DXA, one advantage of QCT is the capacity for separate analysis of the cortical and trabecular BMD. QCT also provides real bone density per bone volume (mg/cm^3). Recently, 3D volume data from the scanning of an entire bone, such as a vertebral body or proximal femur, can be reconstructed to adjust the exact selected region for several serial images in a longitudinal study, which enables monitoring of successive changes with very good precision. QCT-based bone measurements have been used to evaluate age-, sex- and ethnic-related differences in vertebral and femoral geometry and density, providing insights into the development of skeletal fragility.

2.2.1 Volumetric BMD assessment by QCT

CT image is a two step process of initial scan acquisition and then tomographic image reconstruction by a mathematical process of calculating from acquired raw data. All clinical

CT scanners are calibrated to the X-ray attenuation to the water, resulting in CT numbers, measured in Hounsfield Units (HU). To transform HU into bone mineral equivalents (mg/cm^3) an appropriate bone mineral phantom is included in the scan field. QCT is the unique modality that measures the real bone density in a determinate volume (mg/cm^3) without the overlapping of others tissues. QCT differs from DXA as it can allow a selective assessment of both trabecular and cortical bone. Trabecular BMD obtained by QCT shows a more rapid age dependent decrement than that measured by DXA. Single Energy QCT is normally used for clinical setting, though BMD estimation can be altered by quantity of fat tissue, which substitutes the red marrow in elderly people. This effect produces an increasing error of evaluation with the increase of elderly patients. Even if Dual Energy QCT improves the accuracy, nevertheless it uses higher radiation dose and longer scanning times without increasing QCT sensibility in discriminating between healthy and osteoporotic subjects. Over the last decade, technical developments in CT, including multi-detector CT (MDCT) have resulted in images of volumes of tissue being acquired very rapidly, and this has had an impact on QCT in that 3D volume images can be acquired rapidly. Such 3D volumetric QCT enables analysis of the hip, the important site of osteoporotic fracture, which was not feasible with 2D single slices.

2.2.2 Vertebral trabecular QCT assessment

The trabecular BMD, particularly in the vertebra, is metabolically more active and may therefore serve as an early indicator of osteoporosis treatment effect. Vertebral trabecular BMD was demonstrated to have a significant correlation with vertebral fracture. Worldwide, the number of subjects in thoracic and abdominal CT examinations has increased dramatically over the last two decades (McCollough et al., 2009). Several recent studies have shown how it is possible to obtain meaningful QCT BMD values from subjects undergoing thoraco-abdominal CT examinations without the use of a calibration phantom. Such BMD values have a high correlation with BMD values obtained from QCT. These studies demonstrate that it is technically feasible to obtain reasonably accurate BMD values in subjects undergoing thoracic or abdominal CT examinations for other reasons. It is very useful for subjects as it will allow predictions of vertebral fractures without additional radiation exposure (Lenchik et al., 2004). The analysis of BMD at different vertebral levels is necessary because most osteoporotic vertebral fractures are located in the thoracolumbar spine between T4 and L1, with the segments between T7 and L1 most affected (Wasnich, 1996). Osteoporotic fractures of the cervical spine are considered uncommon. The etiology of the striking segmental differences for osteoporotic vertebral fractures is not well explained. Recently we measured the trabecular BMD of thoracic and lumbar vertebrae from 1,031 subjects who had undergone MDCT examination (Hayashi et al., 2011). The vertebral trabecular BMD of both men and women tended to gradually decrease from Th1 to L3 in all age categories (Fig. 1). In relation to vertebral level, L3 had the lowest trabecular BMD among the thoracic and lumbar vertebrae. The correlation of the trabecular BMD among thoracic and lumbar vertebrae was also studied. On the whole, we found that the further the vertebrae were from each other, the weaker were their correlations of the trabecular BMD, and vice versa. This finding indicates that estimating the BMD of distant vertebrae existing beyond the scope of CT images is difficult. For example, if the BMDs of T7 and T12 are estimated using L3 BMD in CT images from abdominal organ examinations, the estimated accuracy of T12 ($r=0.92$) would be better than that of T7 ($r=0.79$) because T12 is nearer to L3

than T7 (Hayashi et al., 2011). That is to say, it may be appropriate to use an arbitrary vertebra as a first approximation for assessing vertebrae which are in the area of predilection for the fracture. If the BMD of one vertebra is known, the BMD of other vertebrae may be estimated using our knowledge of BMD correlations.

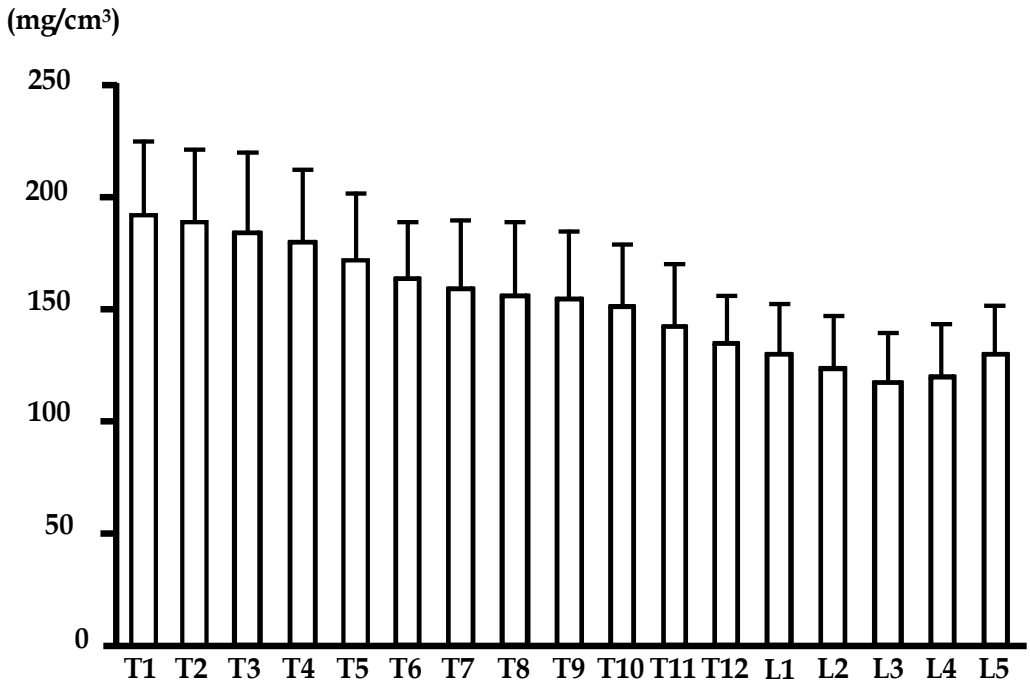

Fig. 1. The trabecular BMD of the thoracic and lumbar vertebrae. The BMD tends to decrease from the first thoracic to third lumbar vertebra (Hayashi et al., 2011)

3. Bone quality assessment

As BMD explains only part of the variation seen in bone strength and only some of the observed reduction in fracture risk that occurs with treatment, recent developments have focused more on measuring bone structure and quality of both cortical and trabecular bone rather than bone mass alone. This is done with the knowledge that a measure encompassing bone quality and structure along with bone mass will provide a better prediction of fracture risk than bone mass alone.

3.1 Conventional X-rays

Conventional radiography is a low-cost, readily available technique with high spatial resolution capable of providing fine bone detail, especially for appendicular skeleton such as the distal forearm and phalanges. It is widely available method, provides a good tissue contrast and has the potential to reflect bone microstructure. Conventional radiography is the first and most important method to identify fractures. The distal radius fractures are almost always identified by standard radiographs, while hip and especially spine fractures may have a difficult detection with important significance in their management, prognosis and therapy.

A more accurate evaluation of lateral chest radiographs routinely executed could lead to the detection of a major number of vertebral fractures and earlier diagnosis of osteoporosis. Although it is ideally suited for use in large population studies, the limitation of radiography is that as a projection imaging technique, it cannot consistently visualize individual trabecula and it depends heavily on the depth of tissues under investigation. Despite these limitations, the trabecular bone properties could be described by texture analysis. Good correlations were found between direct, 3D measures of trabecular architecture and a multiple parameter model, based on 2D texture parameters, such as fractal, statistical and anisotropy measures (Guggenbuhl et al., 2006). With increasing sophistication of structural analysis techniques and an improving ability to acquire high-resolution radiographic detail, interest remains in developing radiography to more precisely evaluate trabecular bone microstructure.

3.2 Multi-detector computed tomography (MDCT)

Computed tomography (CT) is a 3D X-ray imaging technique, which provides positive contrast of mineralized tissues. The image formation process begins with the acquisition of serial radiographic projections over a range of angular positions around the object of interest. The cross-sectional field of view is then reconstructed using established computational techniques. Similar to simple radiography, the reconstructed image intensity values represent the local X-ray attenuation. A material property related to the electron density. Several classes of CT devices are presently used for high-resolution imaging of trabecular and cortical bone microstructure. The multi-detector CT (MDCT) is a clinical CT technique, which is available in most diagnostic imaging departments and thus a dedicated scanner is not required. Since its inception, the number of detector rows on clinical CT units has increased from 4 to the current clinical standard of 64 rows, although 320-row MDCT systems are also commercially available. As expected, MDCT fared less well with trabecular thickness and number because the spatial resolution of all MDCT systems (250–300μm) remains larger than the trabecular thickness of 50 to 200μm (Issever et al., 2010). Nevertheless, structural parameters by MDCT provide a better discriminator of change than DXA. It has been shown that trabecular bone parameters obtained with MDCT correlate with those determined in contact radiographs from histological bone sections and micro-CT (Link et al., 2003). The advantage of MDCT technique is that more central regions of the skeleton such as the spine and proximal femur can be visualized. However, in order to achieve adequate spatial resolution and image quality the required radiation exposure is substantial, which offsets the technique's applicability in clinical routine and scientific studies. High-resolution CT scanning is associated with considerably higher radiation dose compared with standard techniques for measuring BMD. Using clinical imaging in more central regions of the skeleton such as spine and femur, it is still noted that the trabecular bone architecture visualized with MDCT is more a texture of the trabecular bone than a true visualization of the individual trabecular structure.

3.3 Peripheral quantitative CT and high-resolution peripheral quantitative CT

The peripheral QCT (pQCT) with a resolution comparable to that of MDCT has been available since 1990 to examine the peripheral skeleton. pQCT confers a smaller effective radiation dose and is particularly useful for studying cortical bone changes in metabolic bone disorders because the distal radius contains more cortical bone than the vertebral body. As pQCT units use low-power X-ray tubes, these examinations are slow, with a

tendency toward motion artifact. With this limitation in mind, the feasibility of using clinical CT scanners with a dedicated forearm phantom as an alternative to pQCT has been investigated. The cortical and trabecular BMD, and bone geometrical parameters, such as marrow and cortical cross-sectional area, cortical thickness, periosteal and endosteal circumference, biomechanical parameters can be obtained, like cross-sectional moment of inertia, which is a measure of bending strength, polar moment of inertia, indicating bone strength in torsion and stress strain index (SSI). A non-invasive bone strength marker as SSI measured by pQCT, could be significantly correlated with a biomechanical bone strength index, as maximum load at bone failure, assessed by three-point bending test. pQCT can non-invasively determine bone mechanical properties by assessing parameters with accepted prognostic value on bone strength (Kokoroghiannis, et al., 2009). Bridging the clinical need for an imaging modality with lower radiation dose and better spatial resolution is the high-resolution pQCT (HR-pQCT). This can measure trabecular and cortical bone density and bone microstructure with an isotropic voxel of about 80μm. This technique has excellent precision for both density and structure measurements (Dalzell et al., 2009). In 2005, the first published clinical study assessing HR-pQCT found that postmenopausal women had lower BMD, trabecular number and cortical thickness compared with premenopausal women at the distal radius and tibia, although spine and hip BMD was similar. HR-pQCT is a useful modality for assessing changes in cortical and trabecular bone, with a precision of about 2% to 5% (Boutroy et al., 2005). The main limitation of HR-pQCT is that it requires a dedicated scanner, is confined to examination of distal forearm and leg, has some difficultly with registration in the Z plane and should take into consideration the expected difference among individuals of short or long radial or tibial length.

3.4 Micro-CT (μCT)

The earlier conventional tool for assessing trabecular bone architecture was histomorphometry from bone biopsies, which produces a two-dimensional representation of tissue structure, while bone structure is three-dimensional. In recent years, it has progressively been imposed the direct 3D analysis of biopsy specimens imaged by micro-CT (μCT). The most common application of this technology has been the in vitro quantitation of osteoporotic change in trabecular bone architecture. The μCT system has been demonstrated to be the first device able to non-destructively reveal the "real" trabecular architecture and is an X-ray-based technique that provides 3D images of very high spatial resolution below 8 μm. Since μCT allows the depiction of individual trabecula and enables the full characterization of the trabecular network, many investigators have used it to study the trabecular network at different skeletal sites, in direct relation to biomechanical properties or as a "gold standard" for evaluating other techniques, although most of the μCT are limited to ex vivo investigations. Microarchitectural 3D data elaborated by specific software consents to evaluate many metric and non-metric bone structural parameters, such as the bone volume (BV), tissue volume (TV), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), structure model index (SMI), connectivity degree (Conn.D) and degree of anisotropy (DA).

3.4.1 Hamster bone μCT assessment

Age-related bone loss, which is poorly characterized, is a major underlying cause of osteoporotic fractures in the elderly. In order to identify the morphological feature of age-related bone loss, we investigated sex and site (tibia, femur and vertebra) dependence of

bone microstructure in aging hamsters from 3 to 24 months of age using μCT (Chen et al., 2008a). In the proximal tibia and distal femur, the trabecular BV/TV, Tb.N, Tb.Th and BMD increased to a maximum at 6 or 12 months and then declined progressively from 12 to 24 months of age (Fig. 2). Tb.Sp, trabecular bone pattern factor (TBPf) and SMI increased with age. As compared with male hamsters, BV/TV and Tb.N were significantly lower in females at 18 and 24 months of age. Age-related decrease of BV/TV in the vertebral body was less than that of the femoral and tibial metaphyses. In the mid-femoral diaphysis, cortical bone area remained constant from 3 to 24 months of age. Cortical thickness decreased from 12 to 24 months and cortical BMD declined significantly from 18 to 24 months of age. These findings indicate that skeletal site and sex differences exist in hamster bone structure. Age-related bone changes in hamsters resemble those in humans. Hamsters may be a useful animal model to study at least some aspects of bone loss during human aging.

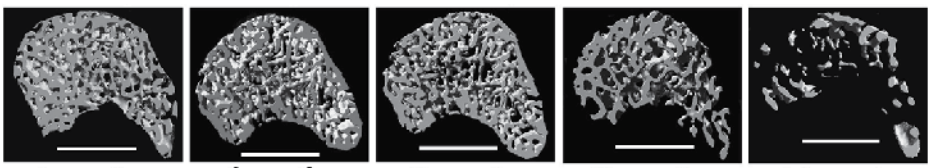

Fig. 2. Three-dimensional images of the proximal tibial metaphysic in female hamsters at 3, 6, 12, 18 and 24 months of age. Trabecular bone volume is highest at 6 and 12 months of age, and declined at 18 and 24 months of age. Scale bar=1.0mm (Chen et al., 2008a)

3.4.2 Osteoporosis model mouse (SAMP6) bone μCT assessment

The senescence-accelerated mouse P6 (SAMP6) is a model of senile osteoporosis, which possesses many features of senile osteoporosis in humans. So far, little is known about the systemic bone microstructural changes that occur at multiple skeletal sites. Recently, we investigated site dependence of bone microstructure and BMD in SAMP6 and the normal control mouse (SAMR1) using quantitative μCT and imaging analysis software (Chen et al., 2009). As compared with SAMR1, the most prominent change in SAMP6 was the reduction of vertebral trabecular BV/TV (Fig. 3) and BMD. Moderate decrease of trabecular bone loss was observed in the proximal tibia and distal femur. Increased bone marrow area and periosteal perimeter were investigated, although the cortical area and cortical thickness had no marked changes in the mid-tibial and mid-femoral cortical bones. These results indicate that bone microstructural properties in SAMP6 are remarkably heterogeneous throughout the skeleton, which is analogous to changes that occur in human bones. These findings further validate the relevance of SAMP6 as a model of senile osteoporosis.

3.4.3 Human vertebral trabecular bone μCT assessment

The vertebral trabecular bone has a complex 3D microstructure, with inhomogeneous morphology. A thorough understanding of regional variations in the microstructural properties is crucial for evaluating age- and gender-related bone loss of the vertebra, and may help to gain more insight into the mechanism of the vertebral osteoporosis and the related fracture risks. Fifty-six fourth lumbar vertebral bodies from 28 women and men (57-98 years of age) cadaver donors were studied. Both women and men were divided into 3 age groups, middle-aged, old age and elderly groups. Five cubic specimens were prepared from

anterosuperior, anteroinferior, central, posterosuperior and posteroinferior regions at sagittal section (Chen et al., 2008b). Bone specimens were examined by μCT and scanning electron microscope. The results showed that BV/TV, Tb.N and Conn.D decreased, while SMI increased significantly between middle-aged and old age groups, and between old age and elderly groups. As compared with women, men had higher Tb.N in the old age group, and higher Conn.D in the middle-aged and old age groups. The central and anterosuperior regions had lower BV/TV and Conn.D than their corresponding posteroinferior region (Fig. 4). Increased resorbing surfaces, perforated or disconnected trabeculae and microcallus formations were found with aging. Vertebral trabeculae are microstructurally heterogeneous. Decreases in BV/TV and Conn.D with age are similar in women and men. Significant differences between women and men are observed at some microstructural paramenters. Age-related vertebral trabecular bone loss may be caused by increased activity of resorption. These findings illustrate potential mechanisms underlying vertebral fractures.

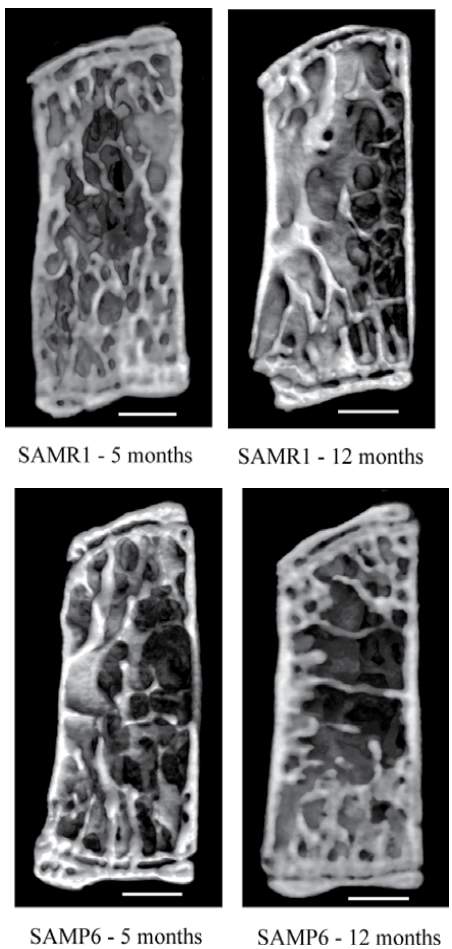

Fig. 3. Micro-CT images representative the fourth lumbar vertebral body in SAMP6 and SAMR1 mice at 5 and 12 months of age. Compared with SAMR1, the trabecular bone is reduced in SAMP6 both at 5 and 12 months of age. Scale bar=0.5mm (Chen et al., 2009)

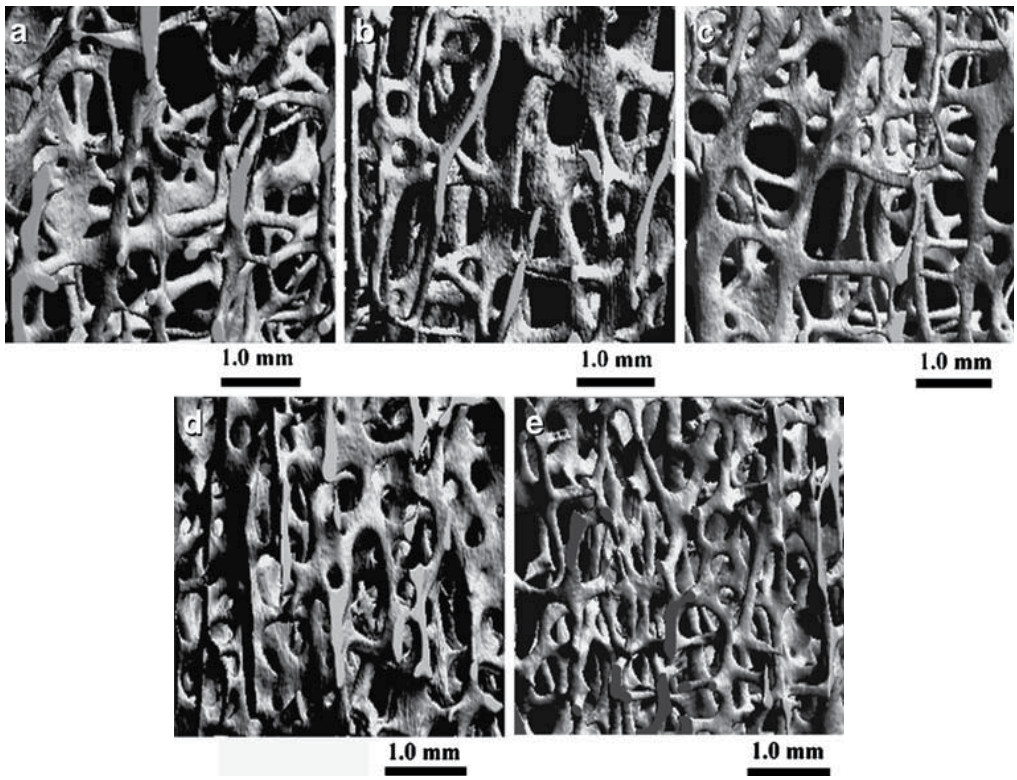

Fig. 4. Three-dimensional micro-CT image in different regions of the vertebral body from a woman aged 78 years: anterosuperior (a), anteroinferior (b), central (c), posterosuperior (d) and posteroinferior (e) regions. The trabecular bone is higher in the posterosuperior and posteroinferior regions than that of the central and anterosuperior regions (Chen et al., 2008b)

3.4.4 Human femoral neck μCT assessment

Femoral neck fracture, which is one of the most common outcomes of age-related and postmenopausal osteoporosis, is a significant cause of morbidity and mortality worldwide. Femoral neck fracture is attributed to both cortical and trabecular bone loss. The relative contribution of femoral neck cortical and trabecular bone to whole bone strength is unclear. We identified 3D microstructural changes of both cortical and trabecular bone simultaneously in human femoral neck from 57 to 98 years of age (Chen et al., 2010). The findings demonstrate that cortical thickness (Ct.Th) decreased by 10-15%, cortical porosity (Ca.V/TV) almost doubled between the middle-aged and elderly groups (Fig. 5).

The trabecular BV/TV declined by around 20% between the middle-aged and elderly groups. The most obvious age-related change in the femoral neck is the increase of Ca.V/TV. The decrease of BV/TV with age is more noticeable than that of Ct.Th. There was a significant inverse correlation between Ca.V/TV and BV/TV for both women and men. As compared with women, men had higher Ct.Th and BV/TV and lower Ca.V/TV. These findings may serve as reference for ethnic comparison with age and gender and may help to gain more insight into femoral neck fracture risk.

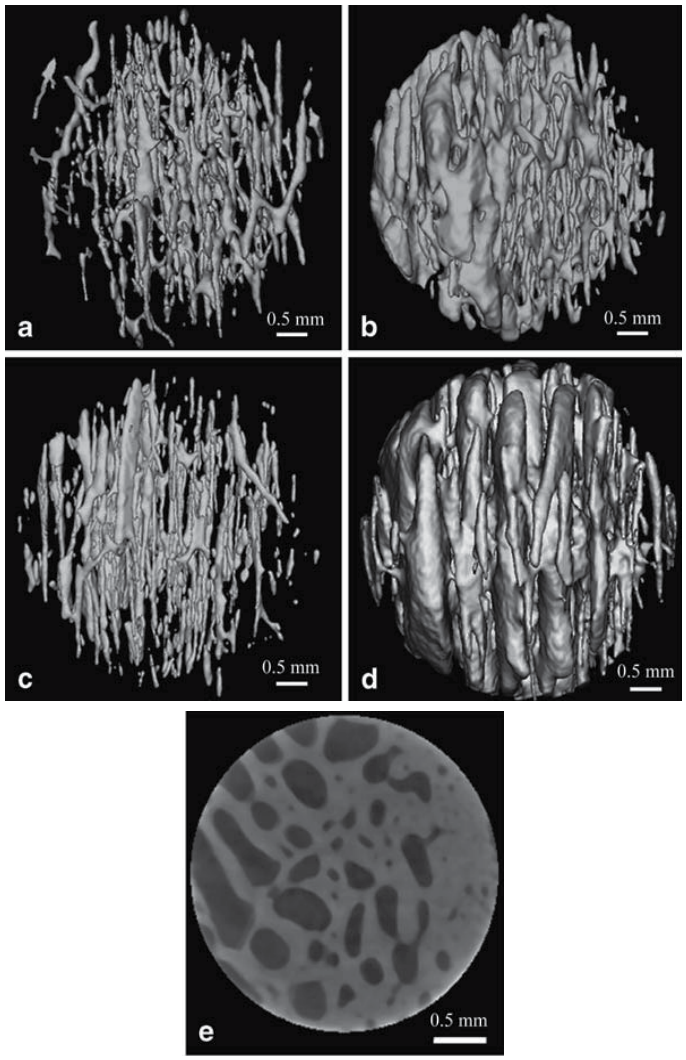

Fig. 5. Three-dimensional reconstructed images of the canal networks in the inferior femoral neck cortex from a man aged 62 years (a), a man aged 92 years (b), a woman aged 62 years (c) and a woman aged 92 years (d). There are more enlarged canals in the 92-year-old woman than that of the 62-year-old man. Representative two-dimensional micro-CT image of the femoral neck cortex from a woman aged 92 years (e) is shown. Periosteal surface faces right for all specimens (Chen et al., 2010)

3.4.5 Human proximal tibia μCT assessment

The analyses of local trabecular microstructure have been mainly performed in regions most susceptible to fractures, such as spine, proximal femur and radius. Studies of the proximal tibia also have an important clinical significance, as it is fractured in aging patients, specifically those suffering from osteoporosis. The proximal tibia, with its rich trabecular network, can be used as a donor site for bone grafting and it is the most easily accessible site for quantification of BMD and bone microstructure. The trabecular bone specimens from the medial

compartment of the proximal tibial metaphyses were examined with μCT and scanning electron microscopy (Chen et al., 2011). It was shown that from 57 to 98 years of age, the trabecular BV/TV decreased by 6-7% and the trabecular BMD declined around 4% per decade at the proximal tibia. Figure 6 shows the typical 3D reconstructions of trabecular bone of the middle-aged and elderly groups for both women and men. The trabecular Tb.Th decreased between the middle-aged and elderly groups similarly in women and men. However, Tb.N decreased by 13% between the middle-aged and elderly groups in women and nearly doubled that in men. As compared with women, men had higher BV/TV and lower Tb.Sp in the old age and elderly groups, and higher Tb.N and Conn.D in the elderly group. Increased trabecular resorbing surfaces, perforated or disconnected trabeculae and microcallus formations were observed with age. These findings indicate that both BMD and BV/TV decreased at the proximal tibia with age similarly for women and men, but significant differences between women and men were observed for some microstructural parameters.

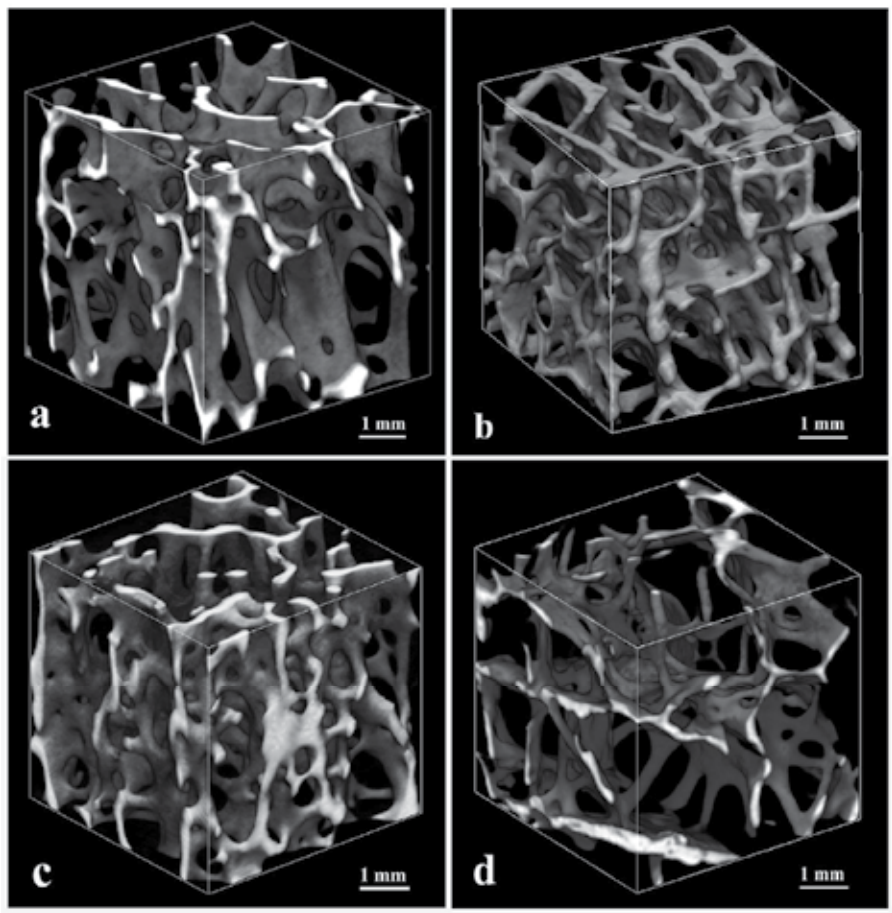

Fig. 6. Three-dimensional reconstructed images of trabecular microstructure at proximal tibia from a man aged 62 years (a), a man aged 92 years (b), a woman aged 62 years (c) and a woman aged 92 years (d). The trabecular bone volume fraction is highest in man aged 62 years and lowest in woman aged 92 years (Chen et al., 2011)

3.5 Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) is a non-ionizing method that uses a strong magnetic field in combination with specialized sequences of radio-frequency pulses to generate high-resolution 3D images of cortical and trabecular bone in vivo. Therefore, it is well suitable for assessing bone structure clinically (Link, 2010). With technical advances in MRI, such as optimized coil design, fast gradients, high gradients and high field strength, MRI scanners provide an in vivo spatial resolution close to the diameter of a single trabecula. MRI signal of trabecular bone itself is not visualized and trabeculae appear as a signal void, surrounded by high-intensity fatty bone marrows. As a result, the bone structure is assessed indirectly via measurements of the surrounding marrow and other soft tissues. Advances in the past decade have focused on image acquisition and analysis techniques to overcome inherent obstacles in MR imaging of bone. With the advent of parallel imaging, motion correlation techniques and new sequences, the limits of spatial resolution and scan time can be further overcome. This non-ionizing, 3D imaging technique is a very attractive tool to analyze trabecular bone structure, investigating bone structure and metabolism in osteoporosis or osteoarthritis. Various studies have been undertaken to optimize image acquisition and post processing, and to calibrate and validate measurements of the trabecular architecture. However, methods can be technically challenging to achieve and optimize.

4. Conclusion

Bone fragility, composite description of bone's biomechanical properties, is directly related to bone's susceptibility to fracture and is inversely related to bone's fracture resistance. As fractures compromise the quality of life and shorten life expectancy, the sophisticated bone imaging modalities play an important role in clearly and accurately identifying the presence and features of fragility fractures. The analysis of bone mass and bone microstructure is an exciting field in the assessment of osteoporotic risk. With the recent advances in MRI and CT, including the introduction of clinical μCT, imaging of true bone structure is becoming more feasible. These non-invasive sophisticated imaging techniques help us to gain more insight into the potential mechanism of metabolic bone diseases, particularly osteoporosis. However, further research is required for improvements in reproducibility, standardization and clinical application of these methods. New technological advances may further refine the imaging of osteoporotic bone and assessment of fracture risk. Recently various computer-aided diagnosis systems were developed for assessment of osteoporosis risks. The dental clinics took numerous panoramic radiographs for examining dental diseases worldwide. Several investigators demonstrated significant associations between mandibular cortical indices on panoramic radiographs and BMD of the skeleton generally, such as the spine and femur, biochemical markers of bone turnover and risk of osteoporotic fractures (Taguchi, 2010). So the computer-aided diagnosis system, based on digital panoramic radiography, may offer a new triage screening for osteoporosis risk in the near future.

5. Acknowledgment

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11

What We Learn from Bone Complications in Congenital Diseases? Thalassemia, an Example

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1. Introduction

The thalassemias, a group of inherited disorders of hemoglobin synthesis, are the most common monogenetic diseases worldwide and are curable by bone marrow transplantation (BMT). Many patients achieve a lifelong disease-free period after BMT. This has focused attentions on disease and treatment complications, for example bone complications. Some of bone disorders occur before and after transplantation and some of them (osteoporosis) and their complications are life threatening. For a better understanding of the bone complications in thalassemia, a brief review of normal bone is required. However, because thalassemia is a curable congenital disease and with an ethical background, the investigation of bone disorders in thalassemic patients (before and after transplantation), can provide a model of calcium and bone metabolism. This model, based on clinical and research findings before and after transplantation, can enlighten factors affecting bone and mineral metabolism throughout the life (disease period and cure period can be considered as periods of bone loss and bone gain through-out the normal life). This model can help in the understanding and management of bone disorders in other bone diseases and in primary osteoporosis. As a resident of a country with a large population of thalassemic patients (Iran), it is author's special interest that such studies help these patients achieve a better quality of life and decrease the burden of this disease not only in Iran but also in other countries worldwide.

2. What is thalassemia

2.1 Disease name and synonyms

The term thalassemia, has two components thalassa (sea) and haima (blood), both from Greek. Beta-thalassemia includes three main forms: thalassemia major ("Cooley's Anemia" or "Mediterranean Anemia"), Thalassemia Intermedia and Thalassemia Minor ("beta-thalassemia carrier", "beta-thalassemia trait" or "heterozygous beta-thalassemia") (Galanello & Origa, 2010). In this review the author's focus is on β-thalassemia major and its bone complications.

2.2 Definition

The thalassemias, hereditary hematologic disorders, are caused by defective synthesis of one or more of the hemoglobin (Hb) chains (Muncie & Campbell, 2009). Hb molecule is a

tetramer composed of 4 -globin polypeptide (2 alpha-globin and 2 beta-globin) plus a heme prosthetic group, to form the complete molecule. In the α-thalassemias, defective production of α-globin chains results in an unstable Hb causes and mild to moderate hemolytic and hypochromic anemia (Sankaran & Nathan, 2010). Beta thalassemia is caused by reduced or absent synthesis of beta globin chains. Hemolysis and impaired erythropoiesis is the result of this imbalance of globin chains. Fatal hydrops fetalis, is seen in cases of Alpha thalassemia major with hemoglobin Bart's (Muncie & Campbell, 2009).

Beta-thalassemia minor (carrier state) patients , are clinically asymptomatic (they are diagnosed, generally accidental by specific hematological features). Thalassemia major patients are severely transfusion-dependent. Thalassemia intermedia patients, ranging in severity from the asymptomatic carrier patients to the severe transfusion-dependent patients (Cao & Galanello, 2010).

Galanello and Origa, in their article (Galanello & Origa, 2010) suggested the following classification:

- Beta-thalassemia
  - Thalassemia major
  - Thalassemia intermedia
  - Thalassemia minor
- Beta-thalassemia with associated Hb anomalies
  - HbC/Beta-thalassemia
  - HbE/Beta-thalassemia
  - HbS/Beta-thalassemia (clinical condition more similar to sickle cell disease than to thalassemia major or intermedia)
- Hereditary persistence of fetal Hb and beta-thalassemia
- Autosomal dominant forms
- Beta-thalassemia associated with other manifestations
  - Beta-thalassemia-tricothiodystrophy
  - X-linked thrombocytopenia with thalassemia

2.3 Epidemiology of thalassemia

The total annual incidence of symptomatic individuals is estimated to be 1 in 100,000 worldwide and 1 in 10,000 in the European Union (Galanello & Origa, 2010). Approximately 5% of the world's population has a globin variant, and only 1.7% has the alpha or beta thalassemia trait. Thalassemia affects men and women equally and occurs in approximately 4.4 of every 10,000 live births. Alpha thalassemia is most common in persons of African and Southeast Asian descent, and beta thalassemia occurs most often in persons of Mediterranean, African, and Southeast Asian descent. The thalassemia trait affects 5-30% of persons in these ethnic groups (Muncie & Campbell, 2009).

2.4 Genetics of thalassemia

The extent of imbalance between the alpha and non-alpha globin chains, relates to the clinical severity of beta-thalassemia. Cao & Galanello suggest that the beta globin (HBB) gene maps in the short arm of chromosome 11, in a region also containing the delta globin gene, the embryonic epsilon gene, the fetal A-gamma and G-gamma genes, and a pseudogene (_B1). Single nucleotide substitutions, deletions, or insertions of oligonucleotides that leads to frame shift, are the majority of mutations. Beta-thalassemia

rarely results from gross gene deletion. In addition to the variation in the phenotype resulting from allelic heterogeneity at the beta globin locus, the phenotype of beta-thalassemia can also be modified by the action of genetic factors mapping outside the globin gene cluster and not influencing fetal hemoglobin (Cao & Galanello, 2010).

2.5 Pathophysiology of thalassemia

Fessas (1963), as cited in Sankaran & Nathan, 2010, described unbalanced globin chain synthesis, as the cause of the b-thalassemia syndromes. Intraerythroblastic inclusions of unpaired a-globin molecules , results disease manifestations (Sankaran & Nathan, 2010).
Galanello & Origa explain two mechanisms for increase the clinical and hematological severity in beta-thalassemia heterozygote patients. In first mechanism, an excess of unassembled alpha chains (resulting in premature destruction of red blood cell precursors) is caused by the coinheritance of both heterozygous beta-thalassemia and triple or quadruple alpha globin gene arrangement, that increases the magnitude of the imbalance of alpha/non-alpha globin chain synthesis. In the other mechanism, premature destruction of red blood depends on the presence of a mutation in the beta globin gene, which causes extreme instability of the beta globin chains and the synthesis of truncated beta chain products (Galanello & Origa, 2010).

2.6 Diagnosis of thalassemia

Most individuals with the thalassemia trait are found incidentally when their complete blood count shows mild microcytic anemia. Hemoglobin electrophoresis with the beta thalassemia trait usually has elevated levels of HbA2.
Individuals with beta halassemia major are diagnosed during infancy. Symptoms appear during the second six months of life. The most common symptoms are pallor, irritability, growth retardation, abdominal swelling, and jaundice. Beta thalassemia intermedia patients (with microcytic anemia, but milder symptoms) have start of disease later in their life (Muncie & Campbell, 2009). Genetic sideroblastic anemias, congenital dyserythropoietic anemias, and other conditions with high levels of HbF (such as juvenile myelomonocytic leukemia and aplastic anemia) are considered as differential diagnosis (Galanello & Origa, 2010).

2.7 Signs, symptoms and complications of thalassemia

Hemolytic anemia, poor growth, and skeletal abnormalities during infancy, are major sign and symptoms of beta thalassemia major (Muncie & Campbell, 2009). Growth retardation, pallor, jaundice, poor musculature, hepatosplenomegaly, leg ulcers, the development of masses from extramedullary hematopoiesis, and skeletal changes (results of the bone marrow expansion) are found in untreated or poorly transfused individuals with thalassemia major (Galanello & Origa, 2010). Thalassemia major patients are diagnosed within the first 2 years and require regular blood transfusions to survive (Sankaran & Nathan, 2010). Iron overload is the result of regular blood transfusions. Complications of iron over load includs endocrine complications (growth retardation, failure of sexual maturation, diabetes mellitus, and insufficiency of the parathyroid, thyroid, pituitary, and less commonly, adrenal glands), dilated myocardiopathy, liver fibrosis and cirrhosis. Patients with thalassemia intermedia present later in life with moderate anemia and do not require regular transfusions. Though the thalassemia intermedia patients, come to medical attention later, may show an extended list of complications like hypertrophy of erythroid

marrow with medullary and extramedullary hematopoiesis and its complications (osteoporosis, masses of erythropoietic tissue that primarily affect the spleen, liver, lymph nodes, chest and spine, and bone deformities as well as typical facial changes), gallstones, painful leg ulcers and increased predisposition to thrombosis. Moderate anemia may be the only sign of thalassemia minor patients and they are in general, clinically asymptomatic (Galanello & Origa, 2010).

2.8 Genetic counseling and prenatal diagnosis in thalassemia

As there is big population of thalassemic patients in some countries and there is high carrier rate for thallassemic mutations in certain populations (explained before in part 2.3), population screening is ongoing in them. Availability of genetic counseling and prenatal diagnosis, makes such screening in these countries more usefull (Cao & Galanello, 2010) and use of prenatal diagnosis may be stressed in such countries (Galanello & Origa, 2010).
Analysis of DNA extracted from fetal cells obtained by amniocentesis (at 15–18 weeks gestation), in high-risk pregnancies in which both members are defined carriers of beta-thalassemia, is possible for prenatal diagnosis. Chorionic villus sampling is useful and is performed at approximately 10–12 weeks gestation (Cao & Galanello, 2010).

2.9 Treatment of thalassemia

Many patients with b-thalassemia, and some patients with severe forms of a-thalassemia, require regular transfusions to survive. In the case of b-thalassemia, this therapy has an important effect on reducing the massive ineffective erythropoiesis and organ infiltration and bone destruction that is seen in β-thalassemia patients that are untreated. (Sankaran & Nathan, 2010). With multiple transfusions, iron overload and organ failure (particularly cardiac iron overload and heart failure) are the leading causes of death (Au, 2011), Therefore, after 10–12 transfusions, chelation therapy (an effective but non-absorbable iron chelator, such as desferrioxamine B (DFO) with a short plasma half-life) is initiated 5–7 days a week by 12-hour continuous subcutaneous infusion via a portable pump (Cao & Galanello, 2010).

2.9.1 Splenectomy

Splenectomy is recommended if the annual red cell requirement exceeds 180-200 ml/kg of RBC (assuming that the Hct of the unit of red cells is about 75%). Symptoms of splenic enlargement, leukopenia and/or thrombocytopenia and increasing iron overload despite good chelation, are considered as other indication for splenectomy (Galanello & Origa, 2010).

2.9.2 Bone marrow transplantation (BMT) in thalassemia

It is explained extensively in part 5.

2.9.3 Therapies under investigation in thalassemia

The potential of new chelation strategies, including combination or alternate treatment with available chelators, induction of HbF synthesis that can reduce the severity of beta-thalassemia by improving the imbalance between alpha and non-alpha globin chains, several pharmacologic compounds including 5-azacytidine, decytabine, butyrate derivatives and gene therapy in the management of beta-thalassemia syndromes are described by Cao and Galanello, Sankaran and Nathan as under investigation therapies (Cao & Galanello, 2010; Sankaran & Nathan, 2010).

2.9.4 Treatment of thalassemia in developed versus underdeveloped countries

In United States and Europe (as developed countries), there are approximately 10,000 homozygous patients with thalassemia. In such countries, due to effective prevention methods, the number of new cases is progressively decreasing. The result of high-quality medical care is longer life expectancy and a relatively good quality of life. BMT and gene therapy, is performed in such countires. The need of Western cultures is to develop improved support for patients with thalassemia and their families (Rund & Rachmilewitz, 2005). In a recent study by Hamidi et al, in Iran, low bone mass was significantly less prevalent in thalassemic patients in comparison to previous studies (Hamidi et al, 2010). Good bone health in patients may also be due to better and developing health network services in Iran and in other countries with high populations of these patients, thus providing a good health service. In Iran there are more than 300 transplanted thalassemic patients (Abolghasemi et al., 2007; Ghavamzadeh, 2009).

The treatment situation of thalassemia patients is different in less developed countries. It is very important because big population of thalassemic patients live there. Safe transfusion and chelation are not universally available. Consequently, many patients with thalassemia in underdeveloped nations die in childhood or adolescence (Rund & Rachmilewitz, 2005).

2.10 Prognosis in thalassemia

Following recent medical advances in transfusion, iron chelation and BMT therapy, prognosis in these patients has improved substantially in the last 20 years. However, the main cause of death in patients with iron overload, remains cardiac disease (Galanello & Origa, 2010).

- As a congenital disease, bone disorders in thalassemic patients are mainly due to bone growth problems and begin in childhood, thus a brief insight into normal bone growth and related matters are discussed in the following section.

3. Normal bone growth

3.1 Normal bone development

Schonau, explains the first phase of bone development so: in development period of embryo, the axial skeleton and extremities are initially in the form of cartilage. The first spontaneous mineralization occurs in the diaphysis. As a result of the activities of osteoclasts and osteoblasts, this mentioned tissue will be replaced by the mature bone matrix. Bones' longitudinal growth take place in the specialized epiphyseal growth plates in which chondrocytes synthesize cartilage matrix, that will be changed to primary and secondary spongiosa in the metaphyseal junction. The growth of The axial skeleton thickness happens due to periostal and endosteal growth(Schonau, 1998). Turn-over of the bones is necessary for either normal mineralized bone matrix maintenance or bone's growth. In healthy adults, resorption and formation of bones take place together in the remodeling process. Though this process is important for maintaining normal skeletal integrity, it does not have any role in changes in bone shape. Diversely, growth of childhood skeletal takes place in bone modeling, a process in which increased bone mass and changes in bone shape, happens. If bone resorption exceeds bone formation a problem occurred named Osteopenia. Which can occur in 2 different ways. It happens when bone resorption exceeds bone formation or when bone formation diminishes, but resorption is normal. (von Scheven, 2007). When muscular strength and parallel biomechanical usage increase, an increase in cortical thickness and area must be happened. The ratio of cortical thickness to bone

diameter (corticalis index) increases as child grows up (Schonau, 1998). The velocity of increase of bone density in children, mostly mimics height growth velocity. It means, a first gradual phase of bone acquisition happens in early childhood and a more accelerated phase of accumulation, approximately 8% per year, occurs during adolescence. (von Scheven, 2007). A decrease phase of bone density happens after 20 or 30 years old, before that, bones mass increases to peak bone mass (PBM). Schonau, suggests that the percentage of ash weight of the individual skeletal sections however does not change significantly with age. On the other hand, physiological content of mature bone tissue (matrix plus minerals) does not change essentially with age and represents a kind of "constant." Morogulis (1931) as cited in Schonau, 1998 , also showed that the calcium and phosphate contents of the of very different animal species's skeletal systems were nearly the same . In contrast the water content does change. Up to years 20 the water content in bone tissue decreases . It is because of the high vascularity of the bones during the elevated phase of remodeling and modeling processes in growth time. It decreases later. (Schonau, 1998).

3.2 How peak bone mass is gained

The increase of total skeletal calcium(from approximately 25 g at birth to 900 and 1200 g in adult females and males, respectively), is gotten through bone growth, modeling and remodeling, which proceed at different rates at various skeletal sites. (Rabinovich, 2004). During childhood and adolescence, changes in size and shape of the skeleton happens together. And also bone grow up in width and and cortical thickness. Genetic, hormonal and environmental factors influence all these processes. (Bianchi, 2007). Bone mass increase is faster in adolescence , 25% of the PBM acquired during the two-year period close to peak height velocity. Rabinovich suggests that maximal rates of bone mineral accrual lag behind peak height velocity by 6–12 months, resulting in relatively undermineralized bone and increased fracture risk in the peri-pubertal years. At peak height velocity, males and females have reached 90% of their adult stature but have acquired only 57% of their adult total body bone mineral content (BMC). Bone mineral accrual continues after linear growth is complete, but the timing of PBM remains debatable (Rabinovich, 2004). About 85% of human skeleton is cortical bone and 15% is trabecular. The bone gain and loss during growth or in later age affects these 2 parts, in different ways. Hormonal/metabolic factors influence strongly the trabecular bone density througout the sexual maturation. Cortical bone consolidates slower. Bianchi states that Although the timing of peak values has not been precisely determined, the PBM is probably reached at the end of the second decade in the axial skeleton (predominantly trabecular bone), but only later in the appendicular skeleton (predominantly cortical bone) (Bianchi, 2007). Rabinovich says that is suggested that, though at least 90% of PBM is achieved by age 18, 5–12% of bone mineral density is reached during the third decade (Rabinovich, 2004). Heritable factors is supposed to attribute to approximately 60–80% of the variations in peak bone mass(Bachrach, 2001), Bianchi results that these changes are not only continuous, but also subject to great individual variation, mostly related to the variability of pubertal development, and this is essential for the correct evaluation of BMD in young subjects (Bianchi, 2007).

3.3 Gender differences

Rabinovich explains difference between girls and boys in growing bone: during puberty, estrogen in girls inhibits periosteal formation while stimulating endocortical bone

formation, thus limiting the medullary space. In contrast, in boys, androgens stimulate periosteal formation, bone diameter, and cortical thickness (Rabinovich, 2004). Also, van Kuijk suggests that, both the starting age of the pubertal spurt and the growth process happens earlier in girls, but the duration of the growth spurt and the maximal peak of growth are greater in boys. Increase in bone density starts around the age of 10 in girls and around the age of 12 in boys. (Van Kuijk, 2010).

3.4 Genetics of low bone mass

As Marini and Brandi categorized in their 2010 article (Marini & Brandi, 2010), the main osteoporosis candidate genes are: Calciotrophic and sex hormones and their receptors ((i) Vitamin D receptor (VDR), (ii) Parathyroid hormone (PTH) and PTH receptor (PTHR), (iii) Estrogen Receptor Alpha and Beta (ERα and ERβ), (iv) Calcitonin (CT) and its receptor (CTR), (v) Aromatase (CYP19A1), (vi) Androgen receptor (AR), (vii) Calcium-sensing receptor (CaSR), (viii) Glucocorticoid receptor (GR)), cytokines, growth factors and local regulators ((i) Interleukin-6 (IL6), (ii) Insulin-like growth factor 1 (IGF-I), (iii) Transforming growth factor β1 (TGFβ-1), (iv) Bone morphogenetic protein 7 (BMP7, OP1), (v) Bone morphogenetic protein 4 (BMP4), (vi) Bone morphogenetic protein 2 (BMP2)), Bone matrix proteins ((i) Collagen type I alpha1 (COLIA1), (ii) Collagen type I alpha2 (COLI-A2), (iii) Osteopontin (OPN, SPP1), (iv) Osteocalcin (OCN, BGLAP), (v) Osteonectin (ON, SPARC)) and miscellaneous genes such as (i) Low-density lipoprotein receptor-related protein 5 (LRP5), (ii) Low-density lipoprotein receptor-related protein 6 (LRP6), (iii) Receptor activator of nuclear factor kappa B (RANK), (iv) RANK ligand (RANKL), (v) Osteoprotegerin (OPG), (vi) Sclerotin (SOST), (vii) Chloride channel 7(CLCN7) and (viii) Methylenetetrahydrofolate reductase (MTHFR) (Marini &Brandi, 2010).

3.5 Low bone mass in pediatrics

Congenital connective tissue disorders such as osteogenesis imperfecta and Ehler–Danlos syndrome are important causes of pediatric osteoporosis. Neuromuscular disorders (cerebral palsy and Duchenne muscular dystrophy), childhood cancer, endocrine disorders (Turner Syndrome and juvenile diabetes mellitus), and inborn errors of metabolism (Gaucher disease) and chronic diseases like thalassemia are secondary causes of pediatric osteoporosis include. Don't forget pharmacological treatment, that are used for treatment of common pediatric conditions (iatrogenic causes. Among these, Glucocorticoids and anticonvulsants are known causes. Some add various forms of chemotherapy to this list (Bogunovic et al., 2009). However idiopathic juvenile osteoporosis is an acknowledged cause of osteoporosis in children and may it is the cause of a higher than expected prevalence of inadequate BMD in the pediatric population.

3.6 Problems with DXA in pediatrics

Bone density measurement by dual energy X-ray absorptiometry (DEXA) the standard method for bone mineral Densitometry. It is also one of most non-invasive techniques for the assessment of bone mass (Hamidi et al., 2008). Not surprising, it is used for many pediatric studies that produced many papers in the field of bone densitometry and in body composition (Van Kuijk, 2010). The WHO based the diagnosis of postmenopausal osteoporosis on the presence of a BMD T-score of 2.5 or greater below the mean for young women (Hamidi et al., 2008). The term "low bone mineral density for age" was mentioned

at the "2007 ISCD Pediatric Position Development Conference" as a criterion for low bone mass in children, and is described as a child with a Z-score below -2.0. The difference between adult and pediatric criterion for low bone mass is that children have not reached PBM, yet. Instead, a child's Z-score (comparison of BMD of patient to age and sex matched normal children in reference data of pediatric software) must be noticed. (Daniels et al., 2003). However, must not forget that DXA has challenging aspects in pediatrics densitometry. True bone density is defined as BMC (g) divided by volume (cm3). Bogunovic explains that as DXA is a 2-dimentional projectional technique. In DXA , a two-dimensional projection, measures a three-dimensional object, bone. As a result, the BMD measured by DXA is defined as BMC (g) divided by the projected area (cm2) not devided by the projected volume (cm3). As a consequence of this area measurement of density, smaller bones appear to have a lower BMD than larger bones (Bogunovic et al., 2009). Van Kuijk, reminds us that in adults, bone size does not change over time. In contrast, bone size changes in growing children in 3 dimensions. When measuring children using DXA and following them over time, growth is measured more than actual changes in bone density (Van Kuijk, 2010). Another challenge, as Bogunovic believes is that the assignment of DXA Z-scores is dependent on the comparison of the patient's BMD to normative childhood data for age and sex. The wide variation in height, and, therefore, of bone size in children complicates the interpretation of BMD results especially in short children. Longitudinal evaluation of a given patient over time is complicated by the ever-changing size of the growing skeleton. Furthermore, the rates of skeletal growth vary with each bony dimension (Bogunovic et al., 2009). All these problems, pose a question: To Do or Not to Do DXA for the measurement of bone density and fracture risk in children? In response we must remember some points related to DXA, 1) patients are exposed to less radiation when measuring BMD by DXA, which is very important in children, 2) it is less fearful for children (less noisy with no tunnel) 3) DEXA is used worldwide and many pediatric studies have been published in the field of bone densitometry and in body composition studies, by using this method and 4) Studies suggest that bone mass may contribute to fracture risk in childhood (Van Kuijk, 2010). Therefore, the answer may be that carrying out DXA for the measurement of bone density and fracture risk in children, is a helpful method, although, it must be remembered, as Bogunovic reminds us, that bone fragility in children extends beyond a single BMD measurement and is influenced by bone geometry and body size and the diagnosis of osteoporosis requires the presence of both a clinically significant fracture history and low bone mass (Bogunovic et al., 2009).

3.6.1 Special considerations in the comparison of normal children and children with chronic disease, some points on the BMD of chronically ill children

As explained above, the measurement of BMC (g/cm) and BMD (g/cm2) are not only dependent on the mineral density of cortical and spongious bone, but also on the geometric configuration. This situation is of great importance in pediatrics. Schonau concludes that if BMC or BMD measurement results in decreased values for children with short stature (e. g., with "smaller bones"), this does not necessarily describe a mineral deficiency or a mineralization disorder, as is often thought (Schonau, 1998). Wide variation in age at onset and progression of puberty is another problem. It means a wide variation in the age at attainment of PBM. Some diseases, like juvenile arthritis, is thought to delay pubertal onset

and development. As it is believed that one-third to one-half of the total mineralization in the lumbar spine in adult women is accumulated during the 3 years around the onset of puberty. Therefore Rabinovich concludes that, comparing the BMD of a well-grown 13-year-old girl who is in mid-puberty with that of a small pre-pubertal 13-year-old with juvenile arthritis is fraught with problems. She suggests that a DXA scan is not needed to tell who has the lower BMD. The question then is, is the BMD finding in this small pre-pubertal girl normal? (Rabinovich, 2004). As van Kuijk suggested, children with chronic disorders or medication, should never be compared with age-matched reference (normal) values. They should be compared with children with the same maturation status (skeletal age) (Van Kuijk, 2010).

3.7 Fractures in pediatrics

Fragility fractures are raising in pediatric population. This may be due to growing number of chronic disease in this population. It caused the increase of use of DXA in children. Healthy children with frequent fragility fracture, have been the focus of research. This is changing, may be because escalating of children with chronic diseases and fragility fractures. When an atraumatic event, cause fracture, fragility fracture come true. The difficulty looms here because as Bogunovic et al state, in young children especially, distinction between traumatic and atraumatic fractures may prove to be a challenge (Bogunovic et al., 2009). Other side of this problem, appear there, when there are many papers on fractures in childhood, but very few of these focus to identify fragility fractures, and fewer focused on the concept of osteoporosis in the young in relation to fractures. Bianchi reminds us that fractures, especially in infants, and especially if multiple or repeated, may be the consequence of violence and child abuse. However, fractures are common events in children. Landin, 1997, as cited in Bianchi, 2007, estimated that 42% of boys and 27% of girls sustain a fracture between 0 and 16 years of age. The must fractures in them, occurs between 10 and 15 years and forearm is the most common site (Bianchi, 2007). Is low BMD a risk factor for fractures? Bone mass may contribute to fracture risk in childhood (Bogunovic et al., 2009). Adverse reactions to cow milk, low dietary calcium intake, early age at first fracture, asthma and overweight (Goulding et al., 2005), and low physical activity are suggested as risk factors for fractures in children. Others suggested that lower BMD for body size, lower milk intake and lower physical activity related with recurrent fractures (Manias et al., 2006). Carbonated beverages also had some relations to fragility fractures. In children with chronic diseases, no systematically collected data is available. Bianchi reviewed that and suggested that some studies found no significant differences in the fracture rate between patients and controls. In contrast, many studies found an increased fracture risk in children affected by various diseases such as acute lymphoblastic leukemia, cerebral palsy, celiac disease, organ transplantation and glucocorticoids users (Bianchi, 2007).

3.8 Treatment of low bone mass in children

Unfortunately, though general measures (optimizing the intake of calories, vitamin D and calcium; providing appropriate weight-bearing activity; replacing GH or sex steroids; and minimizing doses of glucocorticoids) are recommended for better acquisition and maintaining of bone mass in children, they may not be sufficient to prevent or restore deficits in bone mass. Anti-resorptive agents (eg. Bisphosphonates), found valuable in treating some disorders, such as steroid-induced osteoporosis. In steroid-induced

osteoporosis, increased bone loss also contributes to the deficit, so anti-resorptive agents, seem affective. The ideal is treatment children to improve the failure of bone mineral acquisition, but they are not recommended yet (Bachrach, 2001). However, the use of different anti-osteoporotic agents (anabolic or anti-resorptive) in pediatric patients is not very common or recommended, especially in young children, due to a lack of large and systematic studies and comprehensive data supporting their efficacy or addressing their adverse effects in pediatric patients.

4. Bone and thalassemia

Osteopenia and are observed in 40–50% of beta-thalassemia Major patients, and so osteoporosis can be considered prominent causes of co-morbidity in this population, which significantly increases fracture risk (Gaudio et al., 2010).
Before discussing bone disorders in thalassemia patients, it is necessary to understand normal bone function and remodeling.

4.1 Bone in normal individuals

Voskaridou & Terpos, explain the role of skeleton, bone properties and BMU as so: the skeleton provides mechanical support for the body and is a reservoir for normal mineral metabolism. Bone is an active tissue constantly being remodeled and changing metabolically through the balanced activity of osteoclasts and osteoblasts on trabecular surfaces. On a microscopic level, bone metabolism always occurs on the surface of the bone at focused sites, each of which is termed a bone metabolism unit (BMU) (Voskaridou & Terpos, 2004). Mundy suggests that the sequence is always the same, osteoclastic bone resorption followed by osteoblastic bone formation to repair the defect. The resorptive phase of the remodeling process has been estimated to last 10 days. This period is followed by repair of the defect by osteoblasts attracted to the site of the resorption defect which then presumably proceed to make new bone. This part of the process takes approximately 3 months (Mundy, 1999). After the lacunae are filled with osteoids, Voskaridou & Terpos state that this newly formed matrix is mineralized with hydroxyapatite, giving the BMU tensile strength (Voskaridou & Terpos, 2004).

4.1.1 Osteoclasts

Hodge et al, describe Osteoclasts as multinucleated cells which differentiate from early myelomonocytic progenitors rather than more differentiated monocyte/macrophage progenitors (Hodge et al., 2004). Roodman describes their function as they reabsorb bone by secreting proteases which dissolve the matrix and produce acid that releases bone mineral into the extracellular space under the ruffled border of the plasma membrane of osteoclasts (Roodman, 2004). Voskaridou & Terpos say osteoclastogenesis requires contact between osteoclast precursors and stromal cells or osteoblasts. They say the adherence of osteoclasts to the bone surface is critical for the bone resorptive process, since agents that interfere with osteoclast attachment, such as cathepsin K, block bone resorption (Voskaridou & Terpos, 2004).
Wittrant et al, state the role of colony-stimulating factor-1 (CSF-1), released by osteoblasts, as it stimulates the proliferation of osteoclast progenitors via the c-fms receptor (CSF-1R) and,

in combination with the receptor activator of nuclear factor-κB ligand (RANKL), leads to the formation of mature osteoclasts (Wittrant et al., 2009).
These two molecules are expressed by bone marrow stromal cells, also (Voskaridou & Terpos, 2004). Wittrant et al names the cells of the mononuclear phagocytic lineage including osteoclast progenitors and mature osteoclasts as well as placental trophoblasts, uterine decidual cells, smooth muscle cells, microglia, renal mesangial cells and osteoblasts, as other sites that express CSF-1R (Wittrant et al., 2009). PTH administration in 7-14 first days, to enhances RANKL- and M-CSF–stimulated osteoclast formation and bone resorption was shown in vivo (Jacome-Galarza et al., 2011). Thyroxine, 1,25-dihydroxyvitamin D3, and cytokines that use gp130 as part of their receptor, such as interleukin-6 (IL-6) and oncostatin M, are named as other factors which enhance RANKL expression (Voskaridou & Terpos, 2004).
Rankle/OPG system has a characteristic position in osteoporosis and metabolic bone disease. Osteoprotegerin (OPG), a secreted member of the tumor necrosis factor receptor superfamily, has been identified as an osteoblast-derived regulator of bone resorption . OPG neutralizes RANK that is essential for osteoclast formation and activation (Morabito et al., 2004). Alteration in the Rank/Rankl/OPG system may favors osteoclasts and osteoporosis formation (Toumba & Skordis, 2010).

4.1.2 Osteoblasts

Marie & Kassem explain in detail that bone formation is dependent on the recruitment of a sufficient number of osteoblasts as well as the activity of individual osteoblasts. They suggest that osteoblastic cells are recruited to bone forming surfaces mainly from a group of skeletal stem cells with osteogenic differentiation potential (referred to as skeletal, mesenchymal stem cells (MSC), or stromal stem cells. Some believe that some of these cells are pericytes located on the outer surface of blood vessels and sinusoids in the bone marrow, though, the exact location of mesenchymal stem cells in vivo is still debatable (Marie & Kassem, 2011).
Wnt signaling pathway is named as a key pathway involved in the regulation of bone mass. Johnson et al in an article in 2004, explained that Wnt signaling is also required for a diverse number of developmental events including mesoderm induction, organogenesis, CNS organization and limb patterning. In addition, a number of Wnt's have been implicated in vertebrate skeletal development. For example, there is evidence that Wnt3a, Wnt4, Wnt5a, Wnt5b, and Wnt7a all have important roles in chondrogenesis. Another member of the Wnt family, Wnt9A (formerly Wnt14), can induce morphological and molecular signs of joint formation when inappropriately expressed, indicating that Wnt9A plays a crucial role in the initiation of synovial joint development. Wnt9A expression can also lead to the arrest and reversal of chondrogenic differentiation in vitro (Johnson et al., 2004). We explained before, the PTH role in resorption. PTH also have anabolic effects and Marie & Kassem explain that the anabolic effects of PTH on bone formation are mediated through PTH receptor-dependent mechanisms. PTH enhances osteoblastic cell proliferation and function, extends the lifespan of mature osteoblasts through antiapoptotic effects, enhances Wnt signaling through inhibition of the Wnt antagonist, sclerostin, and enhances the local production of bone anabolic growth factors such as insulin-like growth factor 1 (IGF1) (Marie & Kassem, 2011). Though the differentiation of osteoblasts is less well understood than the differentiation of osteoclasts (Voskaridou & Terpos, 2004), bone morphogenetic proteins (BMPs) are critical factors that stimulate the growth and differentiation of osteoblasts (Marie

& Kassem, 2011). Voskaridou & Terpos name several factors such as Basic fibroblast growth factor (bFGF), Insulin-like growth factors (IGF, type I and II), Transforming growth factors (TGF, beta 1 and beta 2) and platelet-derived growth factor (PDGF) and a number of hormones, such as PTH, thyroxine, oestrogen, cortisol, insulin, and calcitonin, as well as vitamin D, are involved in the regulation of bone metabolism, effecting both progenitors and mature osteoblastic cells and osteoclasts (Voskaridou & Terpos, 2004).

4.2 Bone complications in thalassemic patients

Peculiar mongoloid appearance, caused by enlargement of the cranial and facial bones, combined with skin discoloration, anemia, splenomegaly and some enlargement of the liver were included in the first description of thalassemia by Cooley & Lee (Wonke, 1998). Galanello & Origa explain Skeletal changes include typical craniofacial changes such as bossing of the skull, prominent malar eminence, depression of the bridge of the nose, tendency for a mongoloid slant of the eye, and hypertrophy of the maxillae, which tend to expose the upper teeth (Galanello & Origa, 2010).

Some experts suggest that, the thalassemic anemia and the need for transfusion, as earlier as appear in the disease course, the facial changes are more prominent, and all agree that the disease course changes are only seen or are more prominent in untreated patients or in those with no regular transfusion program (Cao & Galanello, 2010). Tyler et al suggest that the skeletal changes in untreated thalassemia are due to ineffective erythropoiesis and expansion of the bone marrow which affect every part of the skeleton. These changes include osteoporosis, growth retardation, platyspondyly and kyphosis (Tyler et al., 2006). Anemia, hemosiderosis, iron chelation therapy, and associated hormonal disorders, are described as main causes of spinal deformity (Haidar et al., 2011). Salehi et al. , suggest that expanded erythropoiesis occurs at extra-medullary sites, most commonly resulting in a para-spinal mass but occasionally affecting organs containing pluripotential stem cells (Salehi et al., 2004). Tyler et al., state that skeletal dysplasia, predominantly affects the rapidly growing long bones, in particular the distal ulna, causing irregularity and sclerosis of the physeal–metaphyseal junction and causing splaying of the metaphysis. They say , Deferoxamine (DFX) also exacerbates the observed growth retardation. DFX-induced skeletal dysplasia, may cause toxicity, which is associated with visual and auditory impairment (Tyler et al., 2006).

Among the spinal deformities observed, Papanastasiou et al suggest that an increased prevalence of frontal curves was reported of at least, 5° in 67% of patients with TM. However, scoliosis curvatures of more than 10° and less than 14° were observed in 21.7% of examined patients. It seemed that location, direction, and pattern of the curvatures, age of onset, gender, and rate of progression of this type of scoliosis associated with TM, differed those in patients with idiopathic scoliosis. They, in their greater than 10-year study reported that the prevalence of frontal curves of at least 5° in 43 TM patients was approximately 80%. Scoliosis of at least 10° and not more than 19° was revealed in 28% to 35% of patients. The most common scoliosis curve pattern was the S-shaped (right thoracic, left lumbar). The prevalence of scoliosis was not gender related, irrespective of age and curve magnitude. Progression of scoliosis in the 10-year period was only detected in four (12%) of 34 patients with scoliosis of 5° to 14°, a rate much lower than that reported in patients with idiopathic scoliosis. Only one patient (2.9%) developed scoliosis of 65° that progressed to 85°, and no other patient developed scoliosis curves that required bracing or operative treatment. No correlation was shown between scoliosis progression and (remaining) growth potential, curve pattern, gender, or curve

magnitude (Papanastasiou et al., 2002). As Haidar et al., mention around 24% of curves showed spontaneous resolution, this was equally distributed among all but the right thoracic curve patterns. Left lumbar and thoraco-lumbar scoliosis improved at a rate of 22% and 33%, respectively. However, most of the curves showed a magnitude of less than 10°. This remarkable absence of progression and spontaneous resolution in small curves depicts the unique etiology of scoliosis in this hematologic condition. Of note, thoracic kyphosis increased with patient age, whereas lumbar lordosis decreased with age and followed the changes of thoracic kyphosis. The 'junction' thoracolumbar kyphosis increased with patient age, but independently from thoracic kyphosis and lumbar lordosis. However, neither scoliosis magnitude nor progression was correlated to thoracic kyphosis (Haidar et al., 2011).

4.2.1 Bone and joint pain

Bone or joint pain, reported in 34% of participants during the 30 days before enrollment, in one study in all thalasemic patients. Vogiatzi et al., state that 6 percent required prescription pain medication and an additional 12.2% used analgesics as over-the-counter. They report that age, sex, and thalassemia syndrome were all independent predictors of the presence and severity of bone and joint pain and the odds of more severe pain, increased 47% for each 5-yr age increase. 40% of females, but only twenty-eight percent of males complained of recent pain. Bone pain was reported more frequently among b TM participants (40%) compared with b TI and E-b participants (16% and 19%, respectively) (Vogiatzi et al., 2009). As Haidar et al suggested, though arthralgia has been mainly attributed to iron overload or use of iron chelators, back pain is mainly associated with osteoporosis, compression fractures, and intervertebral disc degeneration. They report that in one study by the Thalassemia Clinical Research Network (TCRN), young adults with thalassemia experienced pain comparable to the general population, whereas older adults (aged 35+) experienced greater pain. There was an association between pain and low vitamin D level. (Haidar et al., 2011). Vogiatzi et al report that GH-deficient patients were reported to have more severe bone pain, as did those with a history of medicated heart disease, cirrhosis, or hepatitis C (Vogiatzi et al., 2009).

4.2.2 Intervertebral disc changes

Haidar et al. In an extended report about bone disease and skeletal complications in patients with β thalassemia major, suggest that a significant difference in disc degeneration severity has been demonstrated between TM patients and controls on MRI and radiographs. The pattern of disc degeneration was different in TM patients as they exhibited multilevel disease with all levels of the lumbar spine involved. Although no clear mechanism has been suggested for the development of disc changes in TM patients, an underlying metabolic basis has been suggested. They say that the degeneration of intervertebral discs results, in part, from weakening of the annulus fibrosus. The chelating agent, deferoxamine, commonly used in patients with TM, is thought to deleteriously affect the integrity and strength of the annulus fibrosus fibers. Alternatively, the injurious effect of iron overload is also postulated as a factor (Haidar et al., 2011).

4.2.3 Osteoporosis

Several sensitive techniques are available for the quantitative assessment of the degree of total bone mass. Bone density measurement by dual energy X-ray absorptiometry (DEXA)

of the lumbar spine and femoral neck is recommended as one of the most reliable non-invasive techniques for the assessment of bone mass (Kanis, 1994).
According to the World Health Organization (WHO, 1994), osteoporosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and a consequential increase in fracture risk. The WHO based the diagnosis of postmenopausal osteoporosis on the presence of a BMD T-score of 2.5 SD or greater below the mean for young women (Hamidi et al., 2008). The term "low bone mineral density for age" was mentioned at the "2007 ISCD Pediatric Position Development Conference" as a criterion for low bone mass in children, and is described as a child with a Z-score below -2.0 (Daniels et al., 2003).
In spite of adequate transfusion and iron chelation, Thalassemia-induced osteoporosis (TIO) is seen in 30–50% of TM patients, that can cause substantially compromised quality of life in thalassemic patients (Mamtani & Kulkarni, 2010).

4.2.3.1 Genetics of bone density in thalassemic patients

Voskaridou and Terpos, reported that polymorphism at the Sp1 site of the collagen type Ia1 (COLIA 1) gene (collagen type I is the major bone matrix protein) was found in approximately 30% of TM patients who were heterozygotes (Ss) and in 4% who were homozygotes (SS) for the Sp1 polymorphism. They reported the female to male ratio was 2:1. This means that male patients with TM carrying the Sp1 mutation may develop severe osteoporosis of the spine and the hip more frequently than patients who do not carry this mutation. The COLIA 1 polymorphism has been also associated with reduced BMD in postmenopausal osteoporosis, and predisposes women to osteoporotic fractures (Voskaridou & Terpos, 2004). Marini & Brandi reported a similarity between this finding and genetic findings in non-thalassemic patients (Marini & Brandi, 2010).
A possible beneficial effect of BsmI on patient response to alendronate therapy should be emphasized (Gaudio et al., 2010). Haidar et al report the vitamin D receptor (VDR) BsmI and FokI polymorphisms to constitute risk factors for bone mineral damage, low BMD, and short stature in pre-pubertal and pubertal patients with TM, (Haidar et al., 2011).
As Gaudio et al say, it should be remembered that the pathogenesis of osteoporosis is multifactorial, and includes environmental (diet, lifestyle, and drugs) as well as acquired (bone marrow expansion, hemochromatosis, chelation therapy, hepatitis, deficiency of growth hormone or insulin growth factor I, and hypogonadism) and genetic factors (Gaudio et al., 2010).

4.2.3.2 Altered modeling/remodeling in thalassemic patients

Haidar et al, believe that most acquired factors act mainly through the inhibition of osteoblastic activity. They suggest that histomorphometry studies have revealed that increased osteoid thickness, increased osteoid maturation and mineralization lag time, and defective mineralization are common in TM pediatric patinets (Haidar et al., 2011). Mildly increased resorption found in adult patients with beta TM (Vogiatzi et al., 2010). Baldini et al explain an interesting hypothesis that the chronic request for blood cell production can play a role in the etiology of osteoporosis through overstimulation of the hematopoietic system, increasing the number of osteoclasts and osteoblasts resulting in accelerated bone turnover (Baldini et al., 2010). However, Domrongkitchaiporn et al., suggest that increased resorption may be a cause of hypogonadism in these patients (Domrongkitchaiporn et al., 2003).

4.2.3.3 Gender differences in bone density in thalassemic patients

Some studies support that gender of thalassemic poatients affects not only the prevalence, but also the severity of osteoporosis syndrome in TM. However the results are contradicted and some studies showed no gender differences in patients with TM, when they were hypogonadal (Toumba & Skordis, 2010).

4.2.3.4 Acquired factors contributing to reduced BMD in beta-thalassemia

4.2.3.4.1 Bone marrow expansion

Expansion of hematopoiesis and bone marrow expansion, caused severe bone deformities with marked facial and limb changes that were originally described by Cooley et al in 1927 in untreated thalassemia major patients (Jensen et al., 1998). As Wonke, also suggests, the bone marrow expansion due to ineffective erythropoiesis is a typical finding in patients with TM and is considered a major cause of bone destruction. The commonest sites for extramedullary hematopoiesis are the spleen, liver and chest; less common sites are para-vertebral masses and brain lesions. As the ribs contain hematopoietic marrow at all ages, overactive marrow results in, osteoporosis of the ribs, localized lucencies, cortical erosions, and 'rib within rib' deformities (Wonke, 1998). Mechanical interruption of bone formation, leading to cortical thinning, increased distortion and fragility of the bones, occurs due to Marrow expansion (Voskaridou & Terpos, 2004). Tyler et al. refer to ineffective hematopoiesis as a cause of severe anemia and increased erythropoietin production, resulting in expansion of the bone marrow by a factor of 15 to 30. They suggest that even with an optimal transfusion regimen, the bone marrow remains hyperactive. The appearance of "cob-webbing" in the pelvis is the reason of the expanded bone marrow that destroys the medullary trabeculae with initial cortical and trabecular thinning and subsequent trabecular coarsening (Tyler et al., 2006).
Salehi et al., even reported spinal cord compression that is seen in these patients, which can cause neurologic compromise and is, in part, due to extramedullary hematopoiesis (Salehi et al., 2004).

4.2.3.4.2 Endocrine complications

4.2.3.4.2.1 General

Idiopathic hemochromatosis (Iron overload), are commonly associated with hypogonadism and diabetes, while the other endocrinopathies seen in patients with β-thalassemia major and Iron overload, are less common in them. As Perera et al. suggest, a significant predictor of endocrine failure is the duration of transfusion therapy (Perera et al., 2010). In below, we explain endocrine disorders in thalassemic patients, more extensively, as these disorders are major and important causes of bone complication in thalassemic patients.

4.2.3.4.2.2 Growth failure

Homozygous b-thalassemias, have almost invariably growth retardation. Soliman et al . describe thes changes as significant size retardation that is observed in stature, sitting height, weight, biacromial (shoulder), and bicristal (iliac crest) breadths (Soliman et al., 2009). All studies do not show such results (Cao & Galanello, 2010). Soliman et al., state that after the age of 4 years, the longitudinal growth patterns, display rates consistently below those of normal controls and the bone age is frequently delayed after the age of 6–7 years. Growth retardation becomes markedly severe with failure of the pubertal growth spurt

(Soliman et al., 2009). Though hemosiderosis-induced damage of the endocrine glands is one of the main causes for their growth failure, Cao & Galanello, Muncie & Campbell, Toumba & Skordis and Soliman et al, state that other factors could considerably contribute to the etiology of this growth delay including (i) chronic anemic hypoxia secondary to low hemoglobin concentration (Muncie & Campbell, 2009) (ii) toxicity of desferrioxamine treatment (Cao & Galanello, 2010); (iii) increased energy expenditure due to high erythopoietic turnover and cardiac work; (iv) nutritional deficiencies including calories, folic-acid, zinc, and vitamin A (Soliman et al., 2009); (v) disturbed calcium homeostasis and bone disease (Toumba & Skordis, 2010) (vi) hepatic and pancreatic dysfunction (Soliman et al., 2009).

Perera et al., emphasize that normal stature is rarely attained, even in the well-managed patient. They report the administration of GH in some centers internationally at the judgment of individual clinicians, but the role or response to GH is not clearly understood in these patients and probably has no clear benefit unless GH deficiency is confirmed by formal testing (generally as a consequence of early childhood pituitary failure) (Perera et al., 2010).

4.2.3.4.2.3 Delayed puberty/hypogonadism in thalassemia

Both primary and secondary sexual development are usually delayed in both genders in b-thalassemia major (Vogiatzi et al., 2005). An association between hypogonadotrophic hypogonadism and osteoporosis in adult patients with TM has been reported in the past. Jensen et al (1998), found that hypogonadotrophic hypogonadism is a substantial contributor to the development of osteoporosis. Hypogonadotrophic hypogonadism is the commonest endocrinological complication in β-thalassaemia major and is present in 42% of patients (Jensen et al., 1998). Perera et al. describe the finding in thses patients as menarche is frequently delayed by an average of 1–2 years, breast development is poor and female patients frequently have oligomenorrhoea/amenorrhoea even if menarche occurs. Men frequently have poor or absent virilization, reduced libido and oligo/azospermia. They report both genders less fertile and commonly require reproductive assistance to achieve a successful pregnancy (Perera et al., 2010). Toumba & Skordis explains the complacation as disruption of gonadotrophin production (due to iron deposition in gonadotrophic cells) and delayed puberty and hypogonadotrophic hypogonadism. They say secondary amenorrhea will invariably develop with time, especially in patients poorly compliant with chelation therapy. Also primary is common. Men also develop hypogonadotrophic hypogonadism and secondary gonadal failure. So low testosterone secretion is common. They report also primary gonadal failure due to iron deposition in the testes and ovaries. (Toumba & Skordis, 2010).

Perera et al,, in an overview of endocrinopathies associated with b-thalassemia major, (2010), highlighted the high prevalence of hypogonadism with resultant growth failure and infertility, and suggested the following approach and managent protocol in these patients:

1. Formal surveillance from the age of 10–12 years to identify changes associated with puberty, including the development of primary or secondary sexual characteristics. Consideration of an endocrine consultation in cases of suspected delayed puberty.
2. In adults, in addition to regular clinical review, annual monitoring of gonadotropin levels and sex hormone levels for both men and women should be organized. If clinically indicated, use of appropriate hormone replacement therapy in cases of hypogonadism.
3. Regular monitoring (1–3 times/year) of zinc levels, especially if patient is on deferiprone. In cases of zinc deficiency, supplementation to normal levels would also be

reasonable until further clarification of the relationship between zinc deficiency and hypogonadism becomes available (Perera et al., 2010).

4.2.3.4.2.4 Fertility in thalassemia

Pregnancy reported generally safe if baseline cardiac function is good (Rund & Rachmilewitz, 2005). Psihogios et al., suggested that with optimal therapy, most young adults with homozygous β-thalassemia can achieve reproductive, sexual, and social experiences similar to those of their healthy peers (Psihogios et al., 2002).

4.2.3.4.2.5 Impaired glucose tolerance and diabetes mellitus in thalassemia

There are different reports on the prevalence of diabetes in thalassemia major, however, Holger Cario reported that the prevalence is about 5%, while impaired glucose tolerance is found in up to 27% of patients (Cario et al., 2003).

Immune system activation against pancreatic beta cells in beta-thalassemia patients, is reported and pancreatic iron deposition is considered as factors that triggers the autoimmune response (iron deposisions act as environmental factor) and immune response, in turn, contributes to selective beta-cell damage (Najafipour et al., 2008). Perera et la.,did not report family history as a risk factor in thalassemic patient (Perera et al., 2010). In the study by Najafipour et al., risk factors reported for impaired glucose metabolism were, age, amount of blood transfused and duration of blood transfusion. Because not all of the patients with thalassemia major could be correctly diagnosed by fasting glucose alone, the authors preferred to use the oral glucose tolerance test (OGTT) rather than fasting blood glucose levels (BGLs) for the diagnosis of abnormal glucose tolerance in thalassemic patients (Najafipour et al., 2008).

Duration of transfusion therapy, in some studies was the strongest predictor for the development of diabetes (every decade of transfusion exposure further increasing the odds of developing diabetes by 2.5 times). The fact that diabetes mellitus is generally seen in the 3rd or 4th decade, may be is explainable by these findings.. Perera et al state that it is prudent to begin screening for diabetes after the 1st decade of transfusions (regular 6th monthly or annual) by assessing fasting BGLs followed by a 75-g 2-h OGTT if fasting results are abnormal (Perera et al., 2010).

Glyburide treatment and antidiabetic compounds improve insulin sensitivity. Treatment with basal-bolus insulin therapy is also used in these patients. However must not forget that effective iron chelation may improve glucose tolerance (Perera et al., 2010; Cario et al., 2003). Some believe that HbA1c is not a good tool for measuring glycemic control because of reduced red cell lifespan, ineffective hematopoiesis and frequent blood transfusions (affect the validity of HbA1c results). They propose serum fructosamine as an alternative way of monitoring glycemic control, though there is some limitations in its use. Blood glucose self monitoring and regular pre-transfusion venous blood glucose measurements may be use for measuring glycemic control, as an alternative ways in these patinets (Perera et al., 2010).

4.2.3.4.2.6 Hypothyroidism in thalassemia

The severity of thyroid dysfunction is variable in thalasemic patients and the reports of prevalence are very different. Najafipour et al, reported the prevalence of hypothyroidism in their patients 16%, but found the prevalence of 13% to 60% in different studies of patients with thalassemia. However they believe that milder forms of thyroid dysfunction are much more common in thalassemic patients (Najafipour et al., 2008). Primary thyroid damage (from iron

infiltration) or secondary problems (due to pituitary dysfunction due to hemosiderosis of thyrotroph cells) are reported in these patients. Duration of transfusion therapy, has been the strongest predictor for development of hypothyroidism (Perera et al., 2010).

4.2.3.4.2.7 Short stature in thalassemia

As an important complication of thalassemia major, we discuss short stature in an independent section, not attached to growth failure. Najafipour et al, reported that 49% of thalassemic patients had a height standard deviation score less than -2 and 83% of thalassemic patients had a height standard deviation score less than -1. Normal stature is rare even in optimally treated patients. (Najafipour et al., 2008).
Even in well treated patients, it is prevalent and this is may be due to endocrine disorders, lifestyle, iron overload and high doses of desferrioxamine (DFX) when tissue iron burden is not very high (Ferrara et al., 2002).

4.2.3.4.2.8 Hypopituitarism in thalassemia

Hypogonadotropic hypogonadism occures in a large proportion of patients, because pituitary gland is one of the most vulnerable target organs to the early toxic effects of iron overload(Cao et al., 2011). Pan-hypopituitarism is a rare (especial in patients with good chelation therapy). Perera et al, describe the usual sequence for onset of pituitary dysfunction as begins with FSH, LH, GH and followes by ACTH and TSH (Perera et al., 2010).

4.2.3.4.2.9 The Rankl/OPG system in thalassemia

The increase in RANKL, followed by unmodified OPG levels, with the consequent increase in the RANKL/OPG ratio may represent the cause of uncoupling in bone turnover observed in thalassemia patients (Toumba & Skordis, 2010). Haidar et la, report a negative correlation between 17-b estradiol in female and the RANKL and RANKL and free testosterone in male thalassemia patients. They reason that there is a role for the RANKL/OPG system on the action of sex steroids on bone (Haidar et al., 2011).

4.2.3.4.2.10 GH and IGF1 axis in thalassemia

According to the importance of the GH and IGF1 axis, we investigate this axis in detail in the following:
Despite normal response to provocation, some studies have shown that spontaneous GH secretion is defective in some short patients with TM,. Soliman et al, emphasize that these data means the GH–IGF-I–IGFBP-3 axis in thalassemic children is defective. Structural abnormalities of their pituitary glands is also reported in association with defective GH secretion in thalassemic children. Impaired liver functions (secondary to siderosis and/or chronic viral hepatitis) may cause low IGF-I synthesis. Interestingly, Soliman et al, suggest that increased caloric dietary intake significantly increased IGF-I levels in thalassemic pediatric patinets (Soliman et al., 2009).

4.2.3.4.2.11 Parathyroid gland dysfunction in thalassemia

Hypoparathyroidism is another factor contributes to osteopenia and subsequently osteoporosis. It is believed that this complication develops more in late adolescence A recent study reported a prevalence of up to 13.5% with no sex differences (Angelopoulos et al., 2006 (a)). Main causes of hypoparathyroidism, are iron deposition on parathyroid cells (Galanello & Origa, 2010). Typical biochemical picture of hypoparathyroidism with low calcium and high phosphate levels, is seen in these patinets. Low calcium and phosphorus

are found in 24-hour urine collection. Bone X-rays are characteristic for osteoporosis. Abnormal cerebral CT findings are reported to be related to hypoparathyroidism in thalassemics (Karimi et al., 2009; Angelopoulos et al., 2006 (a)).

4.2.3.4.3 Nutrition, Vitamins, Calcium, minerals and calorie intake in thalassemia

Vitamin C deficiency in iron-overloaded patients, is seen with increases the risk of osteoporotic fractures at the level of ephysial lines (Wonke, 1998). Vitamin D deficiency (although it is not reported in all studies in thalassemic patients) is also implicated in the pathogenesis of osteoporosis in TM patients due to the regulatory effect of vitamin D in both osteoclasts and osteoblasts. Adequate calcium intake during skeletal development can increase bone mass in adolescents (Voskaridou & Terpos, 2004). It was shown that increased caloric dietary intake significantly increased IGF-I levels in thalassemic children. Soliman et al., emphasized that aggressive nutritional therapy and/or GH/IGF-I therapy with vitamin D supplementation and/or calcium may improve bone growth and mineralization and prevent the development of osteoporosis and consequent fractures in these patients. They report that many studies, have also shown that improving caloric intake and supplying micronutrients including vitamin D, zinc, and carnitine have a positive effect on linear growth that can be mediated through increasing IGF-I synthesis (Soliman et al., 2009).

4.2.3.4.4 Liver disease in thalassemia

Liver diseases is a known risk factor for osteoporosis (Toumba & Skordis, 2010). The effect of iron overload in the liver is so huge and prominent that determination of liver iron concentration in a liver biopsy specimen shows a high correlation with total body iron accumulation and is considered the gold standard for the evaluation of iron overload (Galanello & Origa, 2010). Complications of iron overload include involvement of the liver (chronic hepatitis, fibrosis, and cirrhosis) (Cao & Galanello, 2010). Several factors are implicated in the reduction of bone mass in TM as well as liver disease (La Rosa et al., 2005). Growth retardation and short stature in these patients and low vitamin D are described as complications of liver disease (Baldini et al., 2010).

4.2.3.4.5 Iron overload in thalassemia

As described, iron overload causes many complications in thalassemic disease which affect bone. However, there are some bone complications that are related to iron overload directly. Some authors suggest direct iron toxicity on osteoblasts (Origa et al., 2005; Galanello & Origa, 2010). Mahachoklertwattana et al, suggest that iron deposition in bone may impair osteoid maturation and inhibit mineralization locally, resulting in focal osteomalacia (Mahachoklertwattana et al., 2003). Although all studies do not agree with these findings (Domrongkitchaiporn et al., 2003), the mechanism by which iron interferes with osteoid maturation and mineralization is explained by Toumba & Skordis as the incorporation of iron into crystals of calcium hydroxyapatite, which consequently affects the growth of calcium hydroxyapatite crystals and increases osteoids in bone tissue (Toumba & Skordis, 2010). Mahachoklertwattana reported a study on the effect of iron overload on bone remodeling in animals showed decreased osteoblast recruitment and collagen synthesis, resulting in a decreased rate of bone formation. Iron deposits in bone and low circulating IGF-I levels may partly contribute to the above findings (Mahachoklertwattana et al., 2003). Domrongkitchaiporn et al., described extensive iron staining on trabecular surfaces and a marked reduction in trabecular bone volume without significant alteration in bone

formation and bone resorption rates, as well as a significant reduction in BMD in 18 thalassemic patients (Domrongkitchaiporn et al., 2003). Thus, it seems that further studies are needed to address the effect of iron toxicity on bone metabolism in thalassemia.

4.2.3.4.6 Chelation therapy in thalassemia

Chelation therapy is a known risk factor for bone problem in thalassemia patients (Origa et al., 2005; Vogiatzi et al., 2010) Growth failure and bone abnormalities, and cartilage alterations are reported as chelating therapy complication (Toumba & Skordis, 2010). Wonke et al., described the role of desferrioxamine in osteoporosis of thalassemic patients as follows: Desferrioxamine inhibits DNA synthesis, fibroblast proliferation and collagen formation, and may also cause zinc deficiency. Growth arrest and a reduction in growth velocity, difficulty in walking, frequently complain of pain in the hips and lower back is seen in patients who receive inappropriately high doses of desferrioxamine, specially when the iron burden is low, (Wonke, 1998).

4.2.3.4.7 Physical activity in thalassemia

As Haidar et al. Suggest, the association between mechanical stress and bone mass was first recorded by Galileo in 1683, who noted the relationship between body weight and bone size. They say that the low bone mass in TM patients is associated with reduced physical activity due to complications of the disease and overprotective parents, who do not encourage muscle activity (Haidar et al., 2011). However, bone disease management in these patients now includes increased physical activity (Rund & Rachmilewitz, 2005; Haidar et al., 2011; Wonke, 1998; Toumba & Skordis, 2010). What must not forget is that there is some conditions requiring special attention in recommending physical activity like severe heart disease , splenomegaly, and osteoporosis (Galanello & Origa, 2010).

4.2.3.5 Fractures in thalassemia

From self-reporting and a review of medical records, fractures occur in 36% of thalassemic patients, with 8.9% reporting three or more lifetime fractures. Extremity fractures are most common at 33%, followed by back and hip fractures at 3.6% (in one study, 10% of all fractures were reported in the spine, hip and pelvis). Low bone mass, sex hormone replacement therapy, and at least one iron overload-related endocrinopathy, was related to the prevalence of fracture. Multiple fractures are also a problem in TM patients (Haidar et al., 2011). Vogiatzi et al found The cumulative risk of fractures increased almost linearly with age. Overally, they didn't find,sex difference ; though among participants <20 years of age, males were more likely to have a fracture compared with females. Whites participants had reports of fracture rates more than Asian. Other their findings was that spine and femur BMD Z-score and total body BMC were negatively associated with fracture rate. For a 1-SD decrease in spine or femur BMD Z-score, the mean fracture rate increased by 37% or 47%, respectively ($p < 0.001$ for both) (Vogiatzi et al., 2009).

The peak age of fracture was the mid to late 30s. Interestingly, the percentage of subjects who remained fracture-free by the age of 18 years was significantly higher than population estimates of healthy children without hemoglobinopathies. There did not appear to be an increase in fracture prevalence during the adolescent growth spurt or surrounding the initiation of menstruation, as is typically observed in healthy reference cohorts. This may be attributed to anemia which leads to decreased physical activity and fewer opportunities for recreational fractures. There is decreased time available for sports and physical activity as

these patients spend a significant amount of their time at health care centers, or overprotected by parents and caregivers. In summary, these findings confirm that the epidemiology of fractures in TM remains unique, as it is not correlated with risk taking behavior but is mainly due to vitamin D deficiency or low BMD which become more severe with age in this cohort of patients. (Haidar et al., 2011).

4.2.3.6 Management of thalassemia-induced osteoporosis

Prevention is essential for the effective control of this potentially debilitating morbidity in TM. Annual follow-up of BMD, starting in adolescence, is considered crucial. Haidar et al., recommend that Physical activity must always be encouraged and smoking should be discouraged. Adequate iron chelation, adequate calcium and zinc intake in combination with the administration of vitamin D , may prevent bone loss and fractures later and in adulthood. Hypogonadism and its prevension and treatment in thalassemic patients is very important in management of bone complication in thalassemia (Haidar et al., 2011). Despite the aforementioned measures, patients with TM still continue to lose bone mass and require treatment. Hormonal replacement, Calcitonin, Hydroxyurea, Bisphosphonates (clodronate, alendronate, pamidronate, and zoledronic) are used in the management of osteoporosis in thalassemic patients. Calcitonin, may decrease bone pain. (Voskaridou & Terpos, 2004 and Haidar et al., 2011).

Of course, it must be remembered that the use of these agents in pediatric patients is not very common or recommended, especially in young children, due to a lack of large systematic studies and comprehensive data supporting their efficacy or address their adverse effects in pediatric patients.

4.2.3.7 Bone mineral density in adult thalassemic patients

The thalassemic patients live longer now. Therefore, it is necessary to assess the bone problems in adult thalassemic patients. The increased survival of these patients during the last decade is due to regular transfusion associated with adequate iron chelation. Specific bone deformities are more rare but osteopenia and osteoporosis are more common . Low bone mass occurs despite transfusions, effective chelation, calcium, and vitamin D supplementation and hormonal deficiency replacement (Baldini et al., 2010). Though hypogonadism is important in low bone mass in TM patients, it may not be overt. Even in eugonadal women, as a delay of menarche which is common, a subtle deficiency in ovarian function cannot be ruled out (Carmina et al, 2004). Napoli et al demonstrated, at least in women with thalassemia major, that hormone replacement therapy was unable to prevent bone loss. This suggests that several mechanisms potentially contribute to low bone mass. One of these mechanisms may be vitamin D deficiency. It should be noted that TM patients progressively develop iron overload, and it is possible that a deficiency in liver hydroxylation of vitamin D, or in vitamin D absorption, can appear in older thalassemic patients (Napoli et al., 2006). However, all studies are not agree with high prevalence of low Vit-D in thalassemic patinets. Another problem in these patients is GH-IGF1 axis. It was demonstrated that the GH–IGF-I–IGFBP-3 axis in thalassemic children is defective (Soliman et al., 2009) and it is shown that GH is important in adult life and that replacement therapy should not be ignored in adults with hypopituitarism (La Rosa et al., 2005).

Baldini et al., found that the femoral site is more influenced (by biochemical and clinical factors) than the spinal site. (Baldini et al., 2010). Christoforidis et al., suggested that optimal conventional treatment in β-thalassemia major can help to achieve normal bone mass

acquisition. They stated major contributors to this, as the regression of marrow expansion due to regular transfusions, the prevention of endocrine complications following adequate chelation therapy, and the reduction in deferioxamine-induced bone toxicity with the additional administration of deferiprone. As patients with thalassemia are in greater danger of developing predisposing factors for osteoporosis, optimal bone acquisition, comparable to the normal population, is essential in order to reduce future risks of osteoporosis in adult life. They recommend close surveillance with regular screening, preventive intervention and early management of possible endocrine complications are important in order to secure normal bone health. Life prolongation for patients with thalassemia major also requires improvement in quality of life (Christoforidis et al., 2006). In addition, Baldini et al. suggested that transfusion and chelation treatment can prevent bone demineralization only when applied early in childhood (Baldini et al., 2010).

5. Bone and thalassemia after bone marrow transplantation

5.1 General

Osteoporosis is increased in recipients of heart, kidney, lung, and liver transplants (Petropoulou al., 2010). Patients undergone bone marrow transplantation have some difference with other transplant recipients. Their underlying disease, organ dysfunction, age, and the median interval between diagnosis and transplantation is different. That interval is usually shorter for BMT. Kerschan-Schindl et al., conclude that BMT recipients may receive less pre-treatment impairing bone metabolism, experience fewer restrictions in mobility, and have a more normal nutritional status. Additionally, BMT recipients generally receive less subsequent immunosuppressive therapy which may induce osteopenia (Kerschan-Schindl et al., 2004).

Thalassemic patients are in an increased risk of accelerated bone loss and thus osteoporosis, because BMT is a curative treatment for thalassemia, and many patients achieve a lifelong disease-free period after BMT. Several factors inhance bone loss in them, including gonadal failure, prolonged immobility, decreased osteoprogenitor cells, conditioning regimens, vitamin D deficiency, secondary hyperparathyroidism, cyclosporine and high-corticosteroid use for graft-versus-host disease (D'Souza et al., 2006). Though some of them are not uncommon before transplantation (Angelopoulos et al., 2006 (b)).

Many investigators such as D'Souza et al. (D'Souza et al., 2006) and Schulte et al. (Schulte & Beelen, 2004) reported the significant lowering effect of corticosteroids on BMD in transplanted patients. However, their studies were not specifically on pediatric thalassemic patients, and a study by Daneils et al. did not find a statistically significant correlation between glucocorticoid exposure and BMD in transplanted children.

Kerschan-Schindl et al., suggest the amount of bone loss and the pattern of loss are controversial. The amount of bone loss within 1 year after transplantation varied and was approximately 2% for the lumbar spine and 12% for the femoral neck. At 5 years after allogeneic BMT, the lumbar spine BMD was within normal limits, but the femoral neck BMD was decreased; osteopenia was present in 43% and osteoporosis in 7% of patients (Kerschan-Schindl et al., 2004). Schulte & Beelen, demonstrated data of rapid bone loss during the first 6 months after transplantation (5.7% at the lumbar spine and 6.9% to 8.7% at the femoral neck sites) with no further decline between months 6 and 12, and recovery of bone mass during further follow-up (Schulte & Beelen, 2004).

As stated by Klopfenstein et al. in the study by Petryk et al., the incidence of osteopenia was 18% and the incidence of osteoporosis was 16% prior to BMT, which increased to 33% and 18%, respectively, 1 year after transplantation (Klopfenstein et al., 1999). In the study by Schulte et al., the lowest BMD in the femoral neck was seen 24 months after transplantation (Schulte & Beelen, 2004). However, as BMT is the only curative treatment for thalassemia, some investigators showed that the changes in BMD after transplantation may change in a positive direction (Leung et al., 2005).

5.2 Special considerations

5.2.1 Special consideration in children (Short stature)

Short stature is present in a significant number of transplanted thalassemic children. A close correlation between age at transplant and subsequent growth rate has been demonstrated (subjects who received BMT after 7 years of age, failed to achieve their full genetic potential), however, growth impairment in these subjects is due to multifactorial deranged function of the hypothalamic-pituitary-gonadal axis, abnormal hepatic conversion of steroid hormones to their active metabolites and defective hepatic biosynthesis of insulin-like growth factor (IGF-I). It is possible that iron overload is primarily involved in this phenomenon. Chronically transfused, inadequately chelated patients develop hepatocellular injury and late growth failure within the first decade of life. This is followed in adolescence by pubertal failure and dysfunction of various endocrine organs (De Simone et al., 2001).

5.2.2 Special consideration in older recipients

It must be remembered that early experience suggested that the results of transplantation for thalassemia were particularly poor for patients older than 16 years. However, Lucarelli et al., found that when the revised regimen for class 3 pediatric patients was used for older class 3 patients, the results were much improved (Lucarelli et al., 1999). However, Kaste, et al., recommend routine screening of BMD for all alloBMT patients. They suggest that patients should be advised to evaluate all behaviors which adversely affect bone health eg. avoid smoking, limit intake of caffeine and carbonated beverages, establish a weight-bearing exercise regimen after orthopedic consultation, and ensure adequate dietary intake of calcium and vitamin D. Patients should also be treated for other conditions that affect BMD such as hypogonadism and hypothyroidism (Kaste, et al., 2004).

In Iran, there is a large population of thalassemic patients and after Italy, the largest population of transplanted thalassemic patients. Thus, special attention to bone diseases before and after transplantation is necessary in these patients, and such studies may be helpful in improving life quality in affected individuals.

6. What we have learned about bone and thalassemia

The thalassemias, a group of inherited disorders of hemoglobin synthesis, are the most common monogenetic diseases worldwide and these diseases are curable by BMT. Many patients achieve a lifelong disease-free period after BMT. Thus, special attention to bone diseases before and after transplantation in these patients is necessary, and such studies may be helpful in improving life quality in affected individuals. Coping with huge problems related to the main disease and during and after BMT, the provision of a normal and safe

life for these patients is a humanitarian problem. Some special points on the prevention, diagnosis, management and monitoring of bone disease in thalassemic patients are listed below:

- Assessment of bone conditions in thalassemic patients before and after transplantation is ethical and many assessments are routine.
- As a multifactorial disease (ineffective erythropoiesis and bone marrow expansion, endocrine complications, iron overload and iron chelation therapy (deferoxamine), vitamin deficiencies, and decreased physical activity all affect bone in thalassemic patients), the assessment of any of these risk factors and factors effecting them, are grounds for research which can be used to provide a better life for these patients. This is true for bone diseases following BMT.
- As a congenital disease that affects bone from an early age and is completely curable, the assessment of patients in a cohort before and after transplantation, provides an opportunity to investigate factors which affect bone in a positive or negative way, when bone is being destroyed by the main disease and when the main disease is cured.
- Genetic studies provide a way of identifying the genes responsible for low bone mass in non-thalassemic and normal individuals, especially when there are similarities in genes which cause low bone mass in thalassemic patients and non-thalassemic osteoporotic patients.
- It is questionable whether the international criteria for defining osteopenia and osteoporosis are relevant to patients with TM; also the diagnostic methods used for osteoporosis in thalassemic patients are questionable as multiple factors and micro-structural characteristics are involved in the pathogenesis of osteoporosis.
- Progression from childhood to puberty and adulthood in these patients provides ground for extended and ethical research on cohort changes in bone density and bone metabolism between these periods. As screening for low bone mass is ethical and routine in pediatrics and adults, there is a unique opportunity to assess the correlation between the diagnostic criteria for low BMD in adult and children.
- Assessment of the effects of different preventive and treatment methods and drugs on bone and different risk factors that affect bone in these patients.
- With an ethical background for investigating bone problems in thalassemic patients, providing a model of calcium and bone metabolism, and factors affecting this metabolism, throughout the life (in periods of bone gain and bone loss), is possible. This is possible by using clinical and research findings in these patients. As thalassemia is a congenital disease which is also curable, finding ways for understanding and management of bone disease in other bone disorders and in primary osteoporosis is possible.

7. Conclusion

With the expanding number of thalassemia and transplanted thalassemic patients worldwide, a better understanding of bone diseases is necessary to provide a better and safer life for these patients. The findings from these studies can be used in a model to better understand human bone diseases and help in the management of these conditions.

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What's BMD and What We Do in a BMD Centre?

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1. Introduction

The main parts of osteoporosis clinics are BMD (Bone Mineral Densitometry) centers. For increasing our knowledge about osteoporosis we have to increase our knowledge about BMD (Bone Mineral Density), and increasing the knowledge about BMD has a close relationship with realizing the principles and appliances of BMD machines and DXA method. For specific development in a BMD department, we need to know some historical, technical and practical points about these method and machines. In this review, the last developments in this field are suggested, also.

2. General information about BMD and BMD centres

2.1 What we do in a BMD centre?

1. Determine patient's BMD
2. Estimate the risk of fracture (pathologic fracture) in a patient

2.2 Some historical points about dual X-ray absorptiometry

It is very useful to know the history of BMD and DXA devices. The first marketing of this machine was in 1987 and in1994 this method described as gold standard for osteoporosis diagnosis by World Health Organisation (WHO). It means osteoporosis disease, as we know now, was described in 1994 for the first time.(Lukaski, 1993; Kanis, 1994).

2.3 Distribution of BMD devices around the world

As Kanis and Johnel reported in 2005, 9 countries from 20 countries (in Europe), had more than 10 DXA units per million of the population (the European standard). However it is unclear which percent of machines were dedicated in part or in full to clinical research. They conclude that the majority of countries are under-resourced. Inequity of geographical location, is an important problem, which is a known problem in Italy, Spain, Switzerland and the UK. (Kanis & Johnell,2005). However the distribution and utilization of these machines are increasing worldwide. This statistics seems interesting when you know there was almost 183 machines in Canada in 1998, and there was no such device in Prince Edward Island (of Canada) around 1998. In Canada there are almost 600 devices, nowadays. The European standard is 0.11 DXA machine per 10 000 population (Mithal et al., 2009).

Asian audit in 2009, show us a very different picture in Asia. DXA technology is relatively expensive and is not widely available in most developing Asian countries, especially in rural areas. There was only 450 DXA machines in China for a population of 1.3 billion. In Srilanka only 4 machines exist. (Mithal et al., 2009). In 2008, Indonesia had a total of only 34 DXA machines, half of them in Jakarta, for a population of 237 million (0.001 per 10,000 population)(IOF, 2011). One of the most extreme examples is found in India, reportedly, there was only approximately 100 DXA units, located in six cities. This inequity results in long waiting times or long distances to travel or in many cases, no access (Kanis & Johnell,2005) (Fig. 1. And Fig. 2.). With above examples about distribution of these machines around the world, we explain here the formula used for calculating standard requirement of these machines (this formula is calculated according to number of population and prevalence of risk factors in target population).

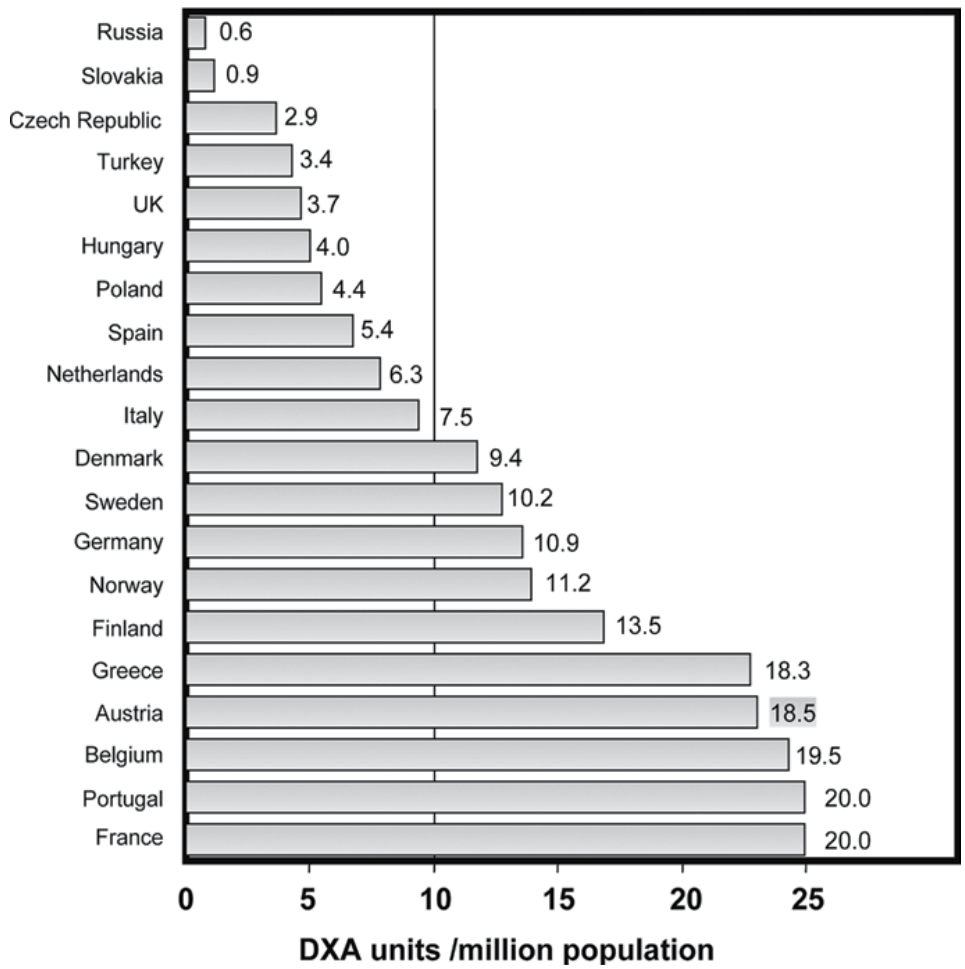

Fig. 1. Density (number /million of the population) of central DXA (spine/hip) units in different European countries in 2003 (from Kanis and Johnell, 2005).

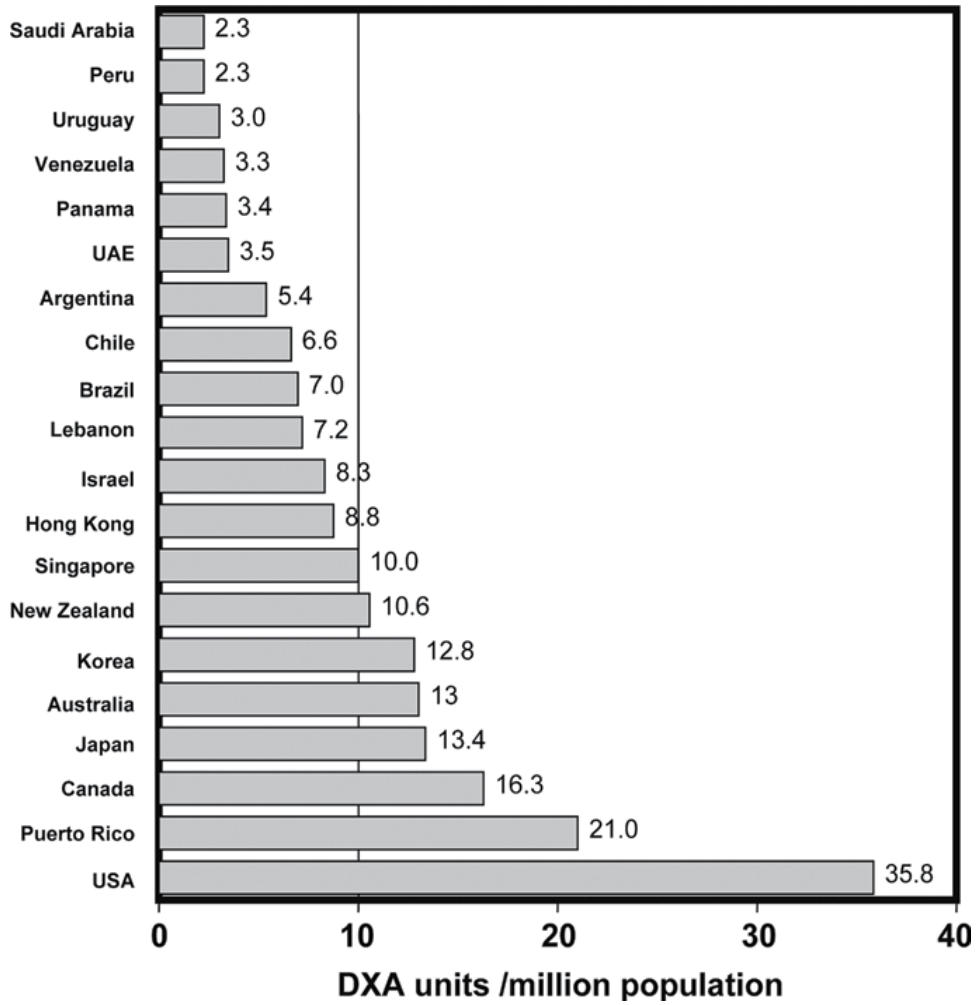

Fig. 2. Density (number /million of the population) of central DXA (spine/hip) units in non-European countries in 2003 (from Kanis and Johnell, 2005).

2.4 Requirement for DXA

Kanis and Johnel, extensively explained the method used for estimation of required number of DXA machines in Europe. As the method is interesting and contained demographic and osteoporotic statistics in Europe, we repeat their explanation as extensive as is used in their article in 2005. Repeat the explanation may be helpful, clearing a guideline for clinicians and researchers, to calculate the requirement of DXA machines in their area or countries. They suggested the requirement for three scenario and in two category, requirements of DXA for risk assessment and requirements of DXA to monitor treatment.

2.4.1 Requirements of DXA for risk assessment

From total population of Europe, it is estimated that, 4 million of them were 65 years old women. The authors assumed that individuals over the 65 years would be tested over the

ensuing 10 years and repeated BMD tests would perform in patient that need treatment or those patients at high risk on the basis of the screening BMD test.
The first scenario (scenario A or screening women with BMD), proposed monitor all women with at the age of 65 years. If the main goal was to measure BMD in all 65 years old women (4.045.000, 65 years old women), this required 3231 DXA units or 4.42 DXA/ million of the total population. In this first scenario, if we assumed that people with the age 66 years and older didn't screen and if they want to screen over a 10- year period, the needs for DXA units would be 6.79/million of total population, giving a total need 11.2 units/million.
The aim of second scenario (scenario B, or clinical case finding with selective use of BMD) was to screen 65 years old women with clinical risk factor referred for DXA at 10 yearly intervals. It means that we sent 65 y/o women for BMD, only when they were high risk for fracture. Finding patients at high risk was based on clinical risk factors. Patients were high risk, when 10-year probability of hip fracture in them (calculated upon risk factors), was 4% and more (This is also called intervention threshold, and the authors considered it a cut-off that treatment is needed for patients). Screening all these patients, need 767 DXA units or 1,05 scan/million of the population. No surprising, the population that need intervention and treatment advances with age. The probability of risk fracture is about 1% at the age of 50 and 52% at the age of 80 years old. Author emphasize that the absolute population size decreases the higher the starting age for testing. They calculated that assessment of women at the older age, (during 10 years period) would require an extra need of 2301 DXA units or 3.16/million of the population (total need for women 65 y/o and older equals to 4.21/million). At younger age, small population is selected for BMD test. So the requirements are not markedly differ by screening policy that starts at age of 50 years. At this age, only nearly 1% of women are selected for treatment. It would be required that more 50,000 DXA tests do for 50 years old women (that add 40 scanning units or 0.05 units/million machines to requirement). After added screened population aged more than 50 years over a 10 year term interval, the total requirement will be 4.5 unit/million. Compare it with 4.21 unit/million required only for screening of 65 y/o women and older.
The third scenario (scenario C, or classic case finding strategy) enlisted only women with strong risk factors for fracture, to do BMD. The authors suggested a different prevalence of risk factors in different age population (29% to 46% depending on age). For testing women of 65 years, 1481 units or 2.03 units/million of the population was required. If BMD considered in women aged more than 65 years and a risk factors prevalence as 46%, 3 million/year over a 10 year interval (30 million, for 10 years) would require testing . This is equal to 3.33 units/million of the population. On the other hand, if BMD tests considered for women aged 50 years or more with one or more these risk factors, BMD testing was needed in 36.9% of the female population aged 50 years or more. Authors calculated this would need 3842 scanning units or 5.3/million of the total population (It seems it is a yearly need, when the whole 10 year need is divided by 10). When only women with incident osteoporotic fracture and aged 65 years or older sent to BMD centers, requirement was 918 scanning visits or 1.3/million of the general population.

2.4.2 Requirements of DXA to monitor treatment

When women referred for treatment, 2 BMD tests may be required. One is at the time of diagnosis, and a second at an interval of 2 years. For scenario B, BMD tests would have been done in 24% of the population at the age of 65 years, some of them do not need treatment

and so don't need a further BMD test in the beginning of treatment (they did't cut the threshold for need to intervention). Additional BMD testing would be required in approximately 10% of women for the purposes of baseline investigation for treatment. If all 65-year-olds were screened, additional pre-treatment BMD tests would equal to 0.4/million scans (322 units) and approximately increase 2-fold after 2 years later. Thus, the steady state requirements would be 966 scanners or 1.33 units/million of the population. Women older than 65 years have a smaller population , but not surprisingly ,a larger proportion would cut an intervention threshold. For example, at the age of 80 years there are approximately 2.15 million women, but with the same test, 73% would be need treatment. But in 50 years old women, approximately 1% of their 5 million population would need treatment. The author emphasized that in women aged 65 years or more, approximately 35% will need treatment and require a BMD tests before and after treatment (2 years later). This gives an annual requirement for 4.6 million scans or 3686 scanning units and a requirement of 5.06/million of the general population. It means for the monitoring of treatment (in 65 y/o women and older), 6.39 uint/million is needed under scenario B. All of these, means the total number 10.6 scanning units/million of the population is needed for assessment plus monitoring of treatment in scenario B (Kanis & Johnell,2005).

2.5 Secular trend of use of DXA

The total number of all older patients performed DXA in the USA has grown up from 501,105 in 1996 to 2,195,548 in 2002. This 4 fold growth during 6 years related to increase the average of lifespan, increase public awareness of osteoporosis and development in therapeutic cares. The maximum application of DXA has been observed in central densitometry. The usage of this method maybe continued for the next few years. However, in some countries, DXA just applied for patients with certain (or specific) risk factors. There are national organization in other countries that prescribe DXA only for patients at multiple risks of osteoporosis. It cause different statistics of use of DXA in different countries (Damilakis et al., 2010).

Results show a great increase in use of bone mass densitometry in Canada. DXA-BMD tests increase 10-fold between years 1993 to 2005, and approximately 500,000 scans perform per year. In Ontario, showed an excessive use of anti-osteoporotic drugs along with the reduction rate of hip and wrist fractures with the increase in BMD test.The growth rate of BMD test appeared to be decreased to 6 to 7% per year. The increase usage rate of BMD-test occurred mainly in 65 years old people or older (Legislative Assembly of Ontario, 2006).

3. Bone densitometry instruments

3.1 Instruments

Lukaski, had a good review of instruments in dual x-ray absorptiometry. Because of its clear and good explanation about the complexity of matter, we mension it here, with almost no change. The first generation commercial dual-energy X-ray absorptiometry (DXA) system became available in 1987 after its initial progress in the late 1960s and 1970s. The three main companies, introduced three X-ray-based absorptiometry systems (approved by the Food and Drug Administration): QDR-1000W3 (Hologic Inc., Waltham, MA), DPX (Lunar Radiation Corp., Madison, WI) and XR-26 (Norland Corporation, Fort Atkinson, WI). Each system uses a source- that generates X-rays at two different energies- a detector and an interface with a computer system.

These three DXA systems operate in different ways. The QDR-1000 and QDR- 1000W systems produce two X-ray beams of different energies by using an X-ray tube alternately pulsed at 70 and 140 kVp peaks. The DPX system uses a constant potential generator and a Cerium K-edge X-ray filtration to generate photons at two energies (40 and 76 keV). The Norland XR-26 unit also employs a constant potential X-ray generator, but it operates at 100 kVp and employs a Samarium filter (K-edge = 46.8 keV). Unlike the DPX and XR-26 systems, the QDR-1000W system has an internal calibration system that consists of a rotating filter wheel composed of three sections (two sections of epoxy-resin-based material consistent with the densities of bone and soft tissue and one section of air). In the QDR system, photons of only one energy are present at any one time, and the detector measures the intensity of the transmitted photons without energy discrimination. An integral line single detector is used in the Lunar DPX system. The XR-26 detector consists of thin and thick sodium iodide crystals (low intensity X rays are stopped by the thin crystal, and high intensity photons are trans mitted and detected by the second thick crystal).

An important advantage of the DXA systems is the increased photon flux emanating from the X-ray sources in comparison to the photon flux from the radioisotope source used in dual-photon absorptiometry. The increased photon flux improves the resolution and precision of the image and reduces the time for a scan. To assess soft tissue composition, the DXA systems use different forms of external calibration. The QDR and XR-26 systems rely on external standards, which are wedges made of aluminum and ucite (polymethylmethacrylate) calibrated against stearic acid as 100% fat, and dilute saline solution as 100% fat-free mineral free tissue. The DPX systems use a plastic polyoxymethylene (Delrin), as 40% fat equivalent and water (~5% fat) as standards (Lukaski, 1993). Recently, the name of Medi-link brand is added to list of machines in FRAX software. Fan beam models are added to DXA machines family and have different beam geometry from pencil beam models. They are explained later.

BMD devices are popular machines, because they are low X-ray radiating, don't need especial preparation for patients and they are not invasive but as it mentioned before, these instruments are not widely distributed in the world, and the expensive cost of these machines is a main reason for it. Properties of these devises that make them expensive are:

- Safety
- The Hardware
- The Software

3.2 Safety

The special method used in these devices, make them low X-ray radiating. They don't need special shielding. We can evaluate the safety of DXA by the radiation dose that each patients or subjects receive. The average skin dose is 1-3 mrad per scan. The radiation dose of DXA is less than other radiologic methods, such as single-photon absorptiometry, dualphoton absorptiometry and quantitative digital radiography, conventional chest x-ray and many others. For example, skin exposures from environmental background are ~3.5 mrad/wk; from dental bite-wing posterior films, 334 mrad and from chest X-ray films, ~8-10 mrad. Thus, we can conclude; for routine measurement of human body composition and bone mineral status , DXA may be noticed a relatively safe method. Manufacturers suggest that it is safe from 1 meter (Lukaski, 1993).

3.2.1 Dose reduction techniques for patients

Damilakis et al, remind us that the system for patients protection against radiation is based on 2 principles: (a) justification and (b) optimisation. Clinically justification of all X- ray exposures used for bone densitometry is very important. Examinations that do not influence patient care, must be avoided.

Preparing patients before bone densitometry is very important. For example metallic things such as jewelry or coins can cause artifact and careful checking for the presence of these items and proper positioning of patient before bone densitometry, will optimize the imaging quality and there will be no need to repeat imaging with additional radiation exposure. In pediatric examinations, proper interaction with the children and parents is essential. All actions should be taken to avoid movement of the child during imaging and to avoid repeating measurement. The duration time of DXA should be minimize and should take into account patient's body size, if possible (Damilakis et al., 2010).

3.2.2 Occupational radiation doses and shielding

Although the annual occupational doses from DXA is very lower than standard occupational radiation dose, but for a pregnant employee that declares pregnancy, special dose reduction should be applied. As Damilakis et al. suggest, The ICRP and European Commission recommend that pregnant individual be protected by the application of a dose up to 1 mGy. Of course, as they emphasize, the exclusion of pregnant workers from DXA examinations on the basis of radiogenic risks from occupational DXA exposure cannot be justified on scientific grounds. Because the scatter radiation can increase the exposure limits for pregnant workers, especially for fan-beam systems. Radiation protection measures should always be taken to ensure that the conceptus dose will be kept below 1 mGy during the declared pregnancy. For monitoring radiation dose, it is recommended to use personal radiation meter at waist level.

Correcting design of the room in which the imaging device has been installed, can influence in limiting the risk of radiation exposure in the workplace. Measurements performed by Larkin et al. as cited in Damilakis et al., 2010, showed that the scatter from fan-beam DXA systems can increase the limits for public exposure i.e. 1 mSv/year. In these cases, additional structural shielding might be required, especially when the distance from the imaging table to the adjacent wall is less than 1 m. They say, parameters like the workload, the material of the walls, the location of the operator and the location and use of rooms that adjoin the imaging room must also be remembered as important factors (Damilakis et al., 2010).

3.3 Hardware

3.3.1 Basic principles of dual-energy X-ray absorptiometry (DXA)

The proportion of beam of X- rays weaken (attenuating) during transporting through a complex material depend on composition of material, the thickness of material and any of its components. Soft tissues, which contain principally water and organic compounds create limitation to the flux (number of X-rays per unit area) of X-rays, and of course, this limitation is lesser than the limitation creates by bone tissue. The un-weakened or un-attenuated energy, in the form of X-ray radiation, is detected by an external detector. In dual-energy X-ray system, there is a source that emits X-rays, which are collimated into a beam (there is a shutter that can turn on and turn off the beam, also). The source lies beneath the patient and the beam transports in a posterior-to-anterior direction, through the body of patient (bone and soft tissue), and goes upward to be detected by a detector, above the patient, lies in the arm of machine (Lukaski, 1993).

3.3.2 Specific technology of dual energy X-ray absorptiometers scanners (DXA)

Before using dual x-ray absorptometry (when single-photon or single-x-ray absorptiometry used), the ROI (region of interest) of scanning, should be immersed in a water bath for densitometry (Fig. 3.). By use of water bath, the water and soft tissue (with almost the same attenuation), make a single compartment of attenuation (on the other hand, the influence of soft-tissue in the measurement significantly reduces and soft tissue don't contributed to measured absorption). They make one compartment and bone makes another compartment with its specific attenuation (than is very different and very higher that other compartment). This can lead to calculating of density of bone, because the attenuation of energy of x-ray beam is related to density of tissue. The density of soft-tissue (and water) is known and constant in almost all humans. The density of bone is not constant and changes one by one. By comparing the attenuation of energy of bone compartment of anyone to attenuation of energy of his soft tissue, machine can calculate the bone density. Without water bath, there is 3 compartment (air, soft tissue and bone), that machine can't separate them exactly and so can't differ between their density, and there is not single reference for comparing density of bone. So finding the exact density of bone would be impossible. Using of water-bath was a development for bone densitometry. But some big practical problems remained. It is practically, impossible to immerse whole body in water bath to measure the bone density of e.g. Spinal region or neck of femur. Water bath was useful for testing BMD of forearm. Remember spine and femur are most important parts of densitometry, because the important or fatal pathologic fracture occurs in these regions and measuring the BMD of e.g. forearm is not a good predictor of BMD or fracture in these important parts. The creating DXA methods, came helpful in solving this big problem. Imagine, using Dual x-ray absorptiometry (using 2 different energy beams) works as water bath in creating two distinguished compartment from compartments that were previously three different compartments of air, soft tissue and bone. The DXA (Dual X-ray absorptiometry) method depends on the differential absorption of two distinct beam energies - a high and low energy beam. When measuring bone, bone will normally have air and soft tissue around it. The high and low energy photons don't change in soft tissue, but the lower energy photon will be significantly reduced by bone tissue (high energy photon don't changes significantly). This difference in reduction of low energy beam, in two different tissue-bone and soft-tissue- can be used for measurement of bone density. On the other hand, the soft tissue component becomes the reference for determining the bone component (Royal Adelaide Hospital, 2009). When two different beams, pass from body compartments, the difference between their intensity before and after passing the soft tissue (and air), don't change (so, the air and soft tissue around the bone create a single compartment). This constant difference can be considered as 1 unit of difference. When two different beams, pass from bone tissue, the low energy beam attenuates significantly after passing bone, it means there is big difference in the intensity of low energy beam before and after passing bone. So the difference between intensity of two high and low energy beams increase significantly and may be multiple times of 1 unit difference reported for soft tissue (and air). This increase in difference is a result of attenuation of low beam energy in bone tissue and relates to bone density. If we have the density of soft tissue compartment, now we can calculate the density of bone. As mentioned before, the density of soft tissue is known and constant and is used as reference for determining bone density in DXA method. It means use of DXA, makes bone densitometry possible, without need to water bath that was needed in single x-ray absorptiomtery. Dual x-ray absorptiometry, makes axial bone densitometry in the conventional form that is performing now, possible (with patient lying on a table in normal atmosphere of an imaging room with no special preparation).

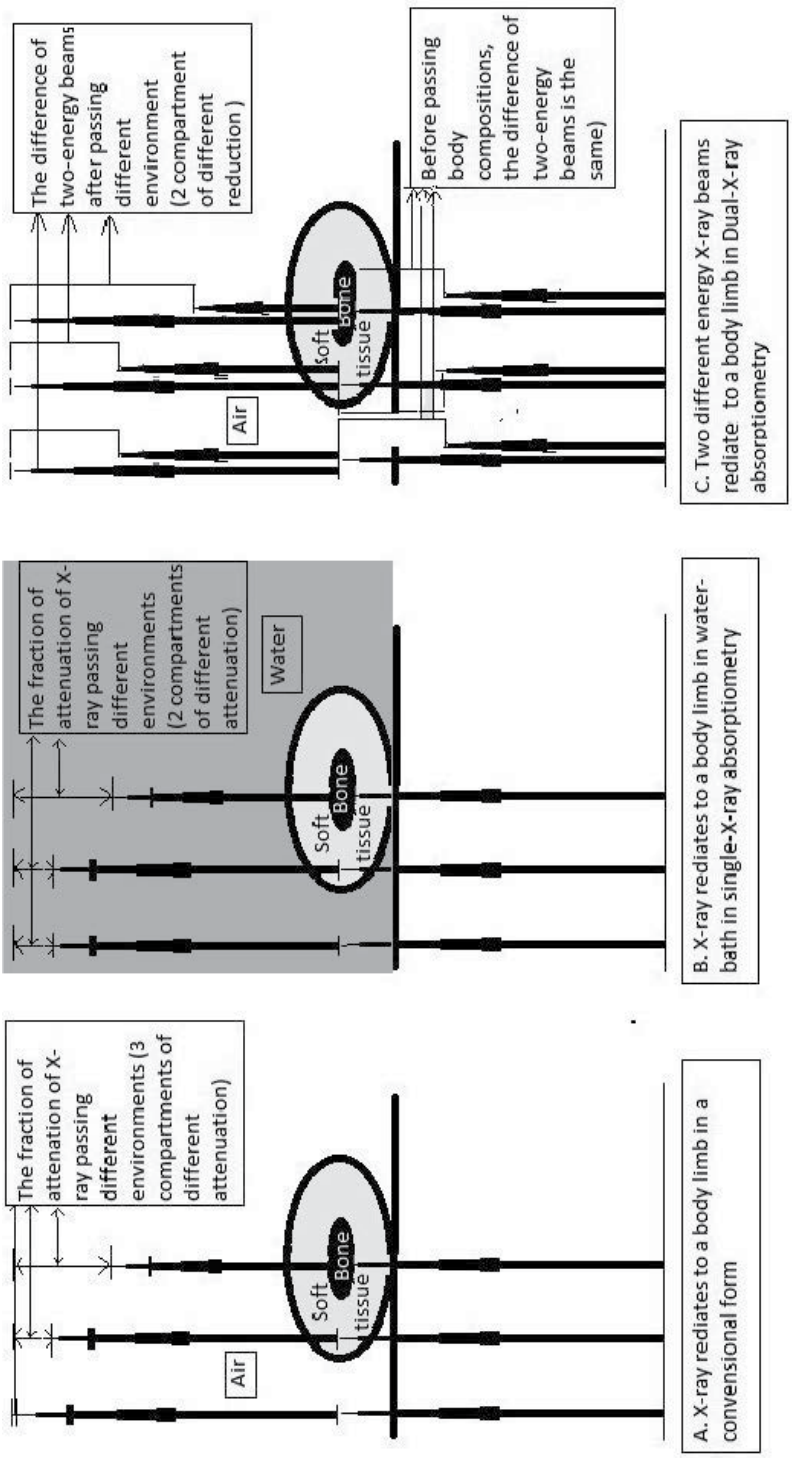

Fig. 3. Different methods of bone densitometry

3.3.3 Quality control

For diagnosing longitudinal changes, assessment of precision error in bone mineral density (BMD) testing is very important (Leslie et al., 2007). Lukaski emphasizes that one parameter of quality control in the use of DXA is the precision of the measurements. Precision is generally reported as the coefficient of variation (CV), which is the standard deviation of repeated measurements expressed as a percentage of the mean of the measurements. The precision of DXA has been assessed for short-term (in vitro and in vivo) and for long-term (in vitro) (Lukaski, 1993).

The International Society for Clinical Densitometry (ISCD) has a standardized methodology for performing an in vivo precision study and recommends that this be performed by each densitometry center. Leslie at al., explain the ISCD procedure as gaining precision error from an assessment with 30 degrees of freedom (df; e.g., 30 subjecs with 2 scans each or 15 subjects with 3 scans each) drawn from the patient of referral population and using the root mean square (RMS) approach (RMS is not explained there) (Leslie et al., 2007).

Lukaski, reports that in first studies, short-term precision and long-term Precision, in different period times and different devices studied. Wahner et al. (1988), as cited in Lukaski; 1993, reported a short-term precision (repeat measurements on the same day) of 0.2 and 0.5% for BMC and BMD, respectively, and a long-term precision (for up to 6 mo) of 0.4% for BMD in lumbar spine phantoms made of hydroxyapatite. Duplicate scans performed on the same day in patients showed a difference of <1% between scans for BMC and BMD. Kelly et al. (1988), as cited in Lukaski; 1993, also observed high reproducibility (CV = 0.23%) of BMD measurements in spine phantoms measured over 6 months. Rencken et al. (1991), as cited in Lukaski; 1993, evaluated the precision of DXA measurements using six different QDR instruments at separate locations. Nine consecutive scans were performed on a single spine phantom at each site. The investigators reported an average precision for BMC and BMD of <1% (range: 0.3-0.6%). The average of the highest and lowest mean values was 1.1% for BMC and 1.07% for BMD. Mazess et al. (1989), as cited in Lukaski; 1993, reported a long-term precision in BMD measurements of 0.6% using a DPX system in a spine phantom over 6 mo. Estimates of 1.8 and 0.9% for the measurement of total body BMC and BMD, respectively, in 12 adults were also reported with a DPX instrument (Mazess et al. 1990, as cited in Lukaski; 1993). Johnson and Dawson-Hughes (1991), as cited in Lukaski; 1993, assessed long-term precision of BMD measurements in six volunteers scanned six times initially and at the same frequency 9 mo later. The short-term precision of BMD measurements in the spine, femoral neck and whole body were 1.08, 2.08 and 0.66%, respectively. The long-term precision was 1.01, 2.07 and 0.62%, respectively. The investigators also reported the precision in determining body composition variables; thus, the precision of whole-body BMC, fat-free mass and fat mass was 0.8, 1.1 and 2.7%, at the start of the study, and 1.2, 1.0, and 1.7%, respectively, after 9 months.

Another aspect of quality control is the accuracy of the DXA measurement. The extent to which DXA measurements represent true bone mineral status has been assessed by measuring the mineral content of cadaver vertebrae of known ash weights and volumes. Ho et al. (1990), as cited in Lukaski; 1993, measured BMC and BMD in lumbar vertebrae from 11 cadavers. The ash weights of 31 lumbar vertebrae and the DXA BMC values were significantly correlated ($r = 0.963$, SEE = 1.01 g; $P < 0.001$). The slope of the regression of ash weight as the dependent variable versus QDR-BMC as the independent variable was 1.0, but

the intercept was 0.59. Although the value of 0.59 was not statistically different from 0, the authors concluded that DXA under estimates ash weight (Lukaski, 1993).
Before to 2000, DXA measurements were conducted with a pencil-beam instrument (Lunar DPX, GE Lunar, Madison WI), and after that a fan-beam instrument was used. As Leslie et al suggested in 2011, instruments were cross-calibrated using anthropomorphic phantoms and 59 volunteers. They say there was no clinically significant differences (T-score differences <0.2). Densitometers showed stable long-term performance [CV<0.5%] and satisfactory in vivo precision (CV 1.7% for L1–4 and 1.1% for the total hip) (Leslie et al., 2011)

3.3.4 The long-term performance of DXA bone densitometers

Monitoring the performance of DXA after long time utilization is very important because any deterioration could change bone mineral density (BMD) measurements and affect clinical management. The importance of DXA in longitudinal trials of new osteoporosis therapies also need constant performance over years to confirm that any altration in bone density is real and not due to machine shifts or fluctuation. In this way, Wells and Ryan, assessed the performance of a 6-year-old bone densitometer (a Lunar DPX alpha), which has undertaken 1500 scans/year over this period. They concluded that the machine performs extremely well over a long period and after 6 years of Performing, measurements is very suitable to be fit for clinical use. It may be can be generalized to all main DXA devices in market (Wells & Ryan, 2000).

3.3.5 Beam geometry

At website of department of nuclear medicine, PET & bone densitometry of Royal Adelaide Hospital (Australia), at section of "Bone Densitometry Equipment", beam geometry of "DXA devices" are explained so:

3.3.5.1 Pencil beam

First generation bone densitometers (isotope and x-ray) use this beam geometry. The photon beam is tightly collimated with one photon source and one detector (some scanners have two, usually photomultiplier tubes). The source and detector are rigidly coupled and moved together in a rectilinear manner to build an image of the bone being examined line by line. The disadvantage of this technology is the relatively slow scan speed (typically 2-4 minutes per scan site). However, the direct relationship between source and detector means that calculated bone and tissue masses are less likely to be artefactual.

3.3.5.2 Fan beam

The second generation of x-ray bone densitometer has a fan geometry, with a source which fans out in the short axis plane of the patient and is measured by an array of detectors in the same plane.
The bones are imaged in one pass along the long axis of the body (as illustrated at middle) providing an immediate advantage in scan speed which is typically about 1 minute on modern scanners.
The disadvantage of fan beam DXA is that the photon flux at the edges is lower than the middle of the image (due to the inverse square law). As a result, mass calculations may have some systematic error, although bone mineral density values have been shown to be unaffected.

3.3.5.3 Narrow fan beam

This is designed to overcome some of the limitations of the fan beam geometry. A small fan beam radiation (about 4cm wide at the detector) in the long axis is detected by an array of detectors. The beam scans the bones in the short patient axis on each individual sweep along the long axis of the patient with some beam overlap. Although slightly slower than a fan beam scanner (1-2 minutes per scan), the mass results should be more accurate as the photon flux has little variability in the area being measured (due to the beam overlap). You can see the schematic figure of different beam geometries in the Fig. 4, from mentioned website (Royal Adelaide Hospital, 2009).

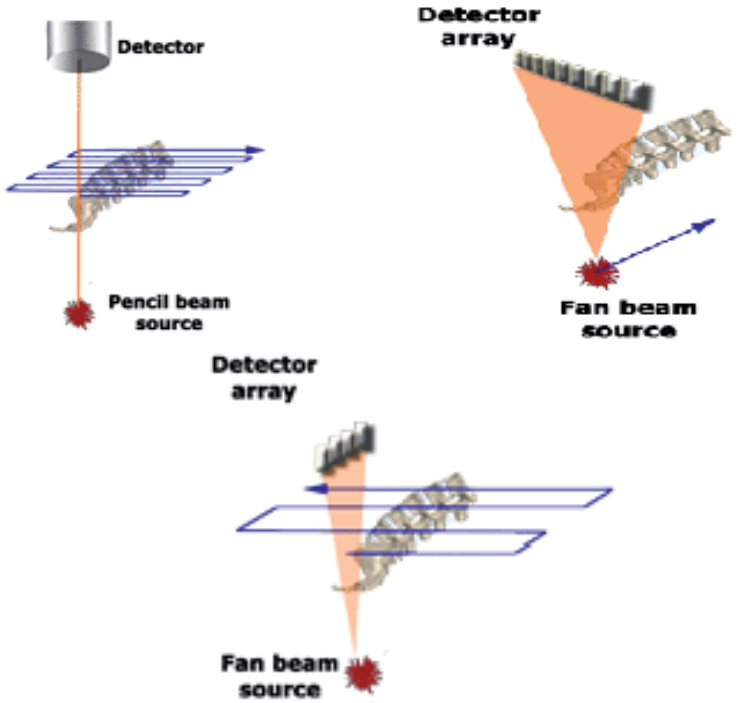

Fig. 4. Beam geometry of DXA mechines (from website of Royal Adelaide Hospital (Australia)

3.4 Software

The reference data of these machines, contain data of BMD tests of almost 5000 Caucasian white normal persons; around 20-80 y/o. Any brand of these machines has different reference data. It is clear that collecting such huge database, nowadays, seems impossible (especially due to cost and financial problems). This makes these method (DXA) and machines, unique. It seems impossible that any other method or brand can replace them in future, at least in near future.

Another ability of the software of this machines is, ability to calculate T-score and Z-score for patients (Shepherd &Blake, 2007):

$$\text{T-score}=\frac{\text{Measured BMD-Young adult mean BMD}}{\text{Young adult population SD}}$$

$$\text{Z-score}=\frac{\text{Measured BMD-Age-matched mean BMD}}{\text{Young adult population SD}}$$

It means after acquisition of absolute BMD of patients by Hardware, the software compute the difference between BMD of patient and young adult mean BMD (from reference data in the software). Then divide it on young adult population standard deviation, contained in the software, the result is T-score. When Z-score is under calculation, the software divides the difference between BMD of patient and age-matched mean BMD and divides it on age-matched population standard deviation. The ability of calculating T—score and Z-score is another interesting characteristic of software of these machines.
As the different brands, have different database, scientists tried to find ways to compare the results of deferent machines. Now we suggest some of these methods.

3.4.1 Providing sBMD

Genant et al, as inventors of sBMD, explained the methods of providing sBMD in their article, so.
We can t compare patient information between various DXA scanners, because there isn't any acceptable universal cross-calibration procedure or standard. Although operating on the same basic principles, normative databases, are specific and different for each scanner. The instruments show differences in scanner design, bone mineral calibration, and analysis algorithms. Lunar and Norland scanners rely on daily scanning of standards to provide a bone tissue equivalent calibration. Hologic uses an internal calibration system, which corrects for short-term instabilities. Also, the software used for analysis of the scans, is manufacturer specific (and unique), especially with regard *to* the edge detection algorithms used for separating bone and soft tissue regions. This implementation causes in variations in the defined bone area (cm2) and bone mineral content (BMC, g) and density (BMD) of the same subject on different systems. Genant et al, study was performed under the auspices of the International DXA Standardization Committee to establish appropriate cross-calibration parameters. Posteroanterior (PA) lumbar spine measurements of 100 women, ages 20-80 years (mean 52.6 ± 16, range of BMD = 0.4-1.6 g/cm2) were obtained on a Norland XR26 Mark II, a Lunar DPX-L, and a Hologic QDR 2000 densitometer using standard procedures (pencil beam mode for all three scanners). Area, BMC, and BMD results from the different scanners were compared for all patients. In addition, the European spine phantom (ESP) and the European spine phantom prototype (ESP prototype), as well as standard phantoms from all three manufacturers, were evaluated on the three systems. To reach universal scanner calibration, they used the intercept and slope of the patient's correlations and the value of the middle vertebra of the ESP as a reference point in a series of standardization formulas, and expressed the results as sBMD (mg/cm2). The correlations of the patients' spinal BMD values were excellent for each of the three scanner pairs. The average absolute difference in patient spinal BMD values (L2-L4) between Hologic and Norland was 0.012 g/cm' (1.3%); it was 0.113 g/cm' (11.7%) between Hologic and Lunar and 0.118 g/cm2 (12.2%) between Norland and Lunar. The phantoms' regression lines approximated those of the patient regression lines, and the phantoms with only one measurement point were very close to the patients' regression lines. After applying the standardization formulas, the average absolute variations for the 100 patients were 28 mg/cm2 (2.7%) for Hologich/Norland, 23 mg/cm2 (2.2%) for Hologic/Lunar, and 29 mg/cm2 (2.8%) for Norland/Lunar. Average BMD results for the patients before correction were 0.972 g/cm2

for Hologic, 1.100 g/cm2 for Lunar, and 0.969 g/cm2 for Norland. After correction, sBMD results for patients were 1045 mg/cm2 for Hologic, 1047 mg/cm2 for Lunar, and 1043 mg/cm2 for Norland. The standardization approach as performed in our study provided compatibility of DXA results obtained on different scanners. Finally the sBMD for different machines calculates as sBMD = 1.0761BMDnorland, sBMDl = 0.9522BMDlunar and sBMDh = 1.0755BMDhologic. (Genant et al., 1994).

3.4.2 Use of NHANES III

When the reference-data of different machines (the young-adult mean BMD), used for defining T-score of patient, the variability within these reference data of different brands, substantially impacts osteoporosis prevalence with using this T-score-based approach. Binkley et al, emphasize that ideally, all bone mass measurement devices would use the same population to define the young-normal mean BMD and SD, a process that cause obtaining of similar T-scores with instruments of different manufacturers. Although use of a single large sample population to develop a unique normative database for all densitometers has been suggested, this process has not been possible. To increase coordination between diagnostic classification, the International Committee for Standards in Bone Measurement (ICSBM) agreed on a universal reference database for the femur based on NHANES III, the only large standardized reference database ever published (Binkley et al., 2005). Looker et al., mention that this data were gathered from 14646 men and women aged 20 years and older, using dual-energy X-ray absorptiometry, and included bone mineral density (BMD), bone mineral content (BMC) and area of bone scanned in four selected regions of interest (ROI) in the proximal femur: femur neck, trochanter, intertrochanter and total. These variables are separated by age and sex for non-Hispanic whites (NHW), non-Hispanic blacks (NHB) and Mexican Americans (MA). They emphasize that the updated data on BMD for the total femur ROI of NHW have been selected as the reference database for femur standardization efforts by the International Committee on Standards in Bone Measurements (Looker et al., 1998). The ICSBM published formulae to convert measured BMD into standardized BMD (of total femur), thereby allowing use of the NHANES III database by other brands' densitometer. The NHANES III data were acquired using Hologic densitometers (Binkley et al., 2005).

4. General consideration in bone mineral densitometry

4.1 Recommendation about ROIs that should assess

Siminoski et al., have some recommendations about ROIs that are under measurement:

- In the lumbar spine, using a minimum of 2 valid vertebra is recommended (if there is problems in L1-L4 vertebrae that cause exclusion one or 2 of them).
- In the proximal femur, Ward's area should not be included in the report, as the small amount of bone yields measurements of poor accuracy and reproducibility.
- If either hip or spine is not valid, forearm BMD is recommended. Preferred site is 1/3 radius, 33% radius or proximal radius.
- When the final report includes a graph of the patient's BMD, it should be based on the same anatomic levels that were used for numeric results; for example if L3 and L4 were excluded from spinal analysis because of degenerative objects, the graph should be based on the combined value for L1 and L2(Siminoski et al., 2005).

4.2 What is the criteria for using other sites for densitometry? Calcaneus an example

For densitometry we can also use appendicular skeleton. Particularly the calcaneus is an excellent site for measurements by a range of techniques. So we use it as an example for describing the rules of choosing ROI for bone mineral densitometry. The calcaneus is easily accessible with little overlying soft tissue. It is not a common fracture site but remember that in the spinal region, the most susceptible sites for fracture are at T7_ T8 and T11_L1, but we measure bone mineral content to L1_L4 because of less overlying soft tissue.

The remodeling of trabecular bone is more active than cortical bone. It means trabecular bone is more active metabolically and more sensitive to metabolic bone changes. Calcaneus is made up, almost entirely of trabecular bone and may provide a more sensitive measurement site for finding early signs of diseases that affect mostly metablism. A number of studies suggested that bone mass of calcaneus may contribute to fracture risk in other sites and that its predictive power is not very different than that of spine and hip. The study by Cummings et al. as cited in Kang and speller; 1999, confirmed this in 65 years old women and over.Interestingly, many early single energy measurements of bone mineral were made in the calcaneus, because it is a peripheral site that can be immersed to water. The arrival of dual energy techniques changed the focus. Earlier studies validated a highly significant correlation between the ashed bone mass of cadaver calcanei and the measured BMC values of calcaneus by densitometry (r=0.97). Kang and Speller, describe calcaneus as a site with excellent accuracy that it's measurements can be made quickly and easily and with portable instruments. (Kang & Speller, 1999)

4.3 Operators, the heart of a BMD center

Correct positioning among other factors is very important to ensure an optimal scan. Simonoski et al., emphasize that correct and consistent positioning and labelling of hip and lumbar spine (as the main job of operators), are important when evaluating serial assessments (monitoring of patients). It is important to follow manufacturer-specific protocols to ensure appropriate comparisons with normative reference data.

Structural abnormalities and artifacts can significantly influence the results. Independent factors, like body weight, may affect BMD results. However, in interpreting the results of a scan, first of all, it must be described whether the scan is valid with regards to positioning, artifact, and analysis, or not (Siminoski et al., 2005)

Fuleihan et al, assessed the effects of the machine, operator and subjects on error of measurements of bone density. They explained their technique for this assessment as an analysis applied to data from a prospective study of BMD measurements on spine phantoms and on pre- and postmenopausal women. Scans performed on the same day or up to 4 weeks apart with DXA (QDR IOOOW, Hologic). Their model assessed (or suggested) that : operators' and subjects' variability were the most causes of errors in measurements rather than machine performance (Fuleihan et al., 1995). Subjects are not changeable or controllable, but operators job can be under quality control and its quality develops by time (and experience). These machines, are not very extensively distributed, and any machine is unique in its way (the data of a second scan of a patients, can be compared to data on the same machine that first BMD is performed, only). These make finding expert operators for these machines, not very easy. What mentioned above, is the cause that operators are called "the heart" of BMD centers. So some-ones believe in this sentence "Never change your operators (in BMD departments) and if the change is inevitable, never change them again."

4.4 Material of a standard BMD report

Shimonoseki et al, recommend that , a standard BMD report should include:

- Patient identifiers.
- DXA scanner identifier.
- BMD results expressed in absolute values (g/cm2; 3 decimal places) and T-score (1 decimal place) for lumbar spine; proximal femur (total hip, femoral neck, and trochanter); and an alternate site (forearm BMD preferred: 1/3 radius, 33% radius or proximal radius) if either hip or spine is not valid.
- A statement about any limitations due to artifacts, if present.
- The fracture risk category (low, moderate, or high). It must be included major clinical factors that modify absolute fracture risk probability (with an indication of the corresponding absolute 10-year fracture risk of <10%, 10-20%, or >20%).
- A statement as to whether the change is statistically significant or not for serial measurements. The BMD centre's least significant change for each skeletal site (in g/cm2) should be included (Siminoski et al., 2005)

4.5 Discordance

Discordance makes difficulties in diagnosis of osteoporosis and management of osteoporotic patients. Moayyeri et al, explain, discordance in diagnosis of osteoporosis that is defined as presence of different categories of diagnosis based on T-score (osteoporosis, osteopenia, and normal) in two skeletal sites of an individual patient. They mansion that discordance has been divided into two groups: major and minor . When the different sites results, are close; i.e., normal in one site and osteopenic in the other site, or, when patient is diagnosed as osteopenic in one site and osteoporotic in the other site, minor discordance happens. When patient diagnosed normal in one site and is osteoporosis in another site, major discordance happens. (Moayyeri et al., 2005). In a clinical study, BMD measurements performed at lumbar spine both for baseline risk assessment and for monitoring purposes. Leslie et al. discuss a difficulty that clinician are confronted with highly discordant measurements and at the same time lumbar spine is worse than femoral neck and about how this should be integrated into the decision-making process. They discuss about different guideline recommendations in this situation. They say under NOF guideline, if t-score in lumbar spine is in osteoporotic range without consideration to estimated risk -by special soft-wares-, treatment should be recommended. In other national guideline such as those from the UK, till a 10 year fracture risk prediction from the femoral neck does not reach the intervention threshold, don't recommend any treatment for patients with osteoporotic lumbar spine. Canadian guidelines have attempted to show the issue of site discordance (in femur) by recommending use of the minimum T-score, in femur for diagnosis and treatment of osteoporosis. However, Leslie et al. suggest that this may systematically overestimates fracture risk and does not consider site-specific differences in fractures or the way BMD declines with age. They suggest that as lumbar spine and hip measurements are both performed for clinical purposes, using a procedure that accurately reflects the contribution of each measurement site to fracture risk, is clearly preferred, so they propose a a procedure for adjusting FRAX probability, based upon the T-score difference between the lumbar spine (LS) and femoral neck (FN). This procedure is termed "offset". They furmulated following rule: "Increase/decrease FRAX estimate for a major fracture by one tenth for each rounded T-score difference between LS and FN." (Leslie et al., 2011)

4.6 Pediatric consideration

4.6.1 Low bone mass in pediatrics

New investigations show prevalence of low BMD in children is very high and it is higher than expected range. Genetic, environmental and iatrogenic factor are 3 most important factor that lead to bone disorders in children.

Bogunovic et al., name causes of pediatric osteoporosis as idiopathic juvenile osteoporosis and heritable connective tissue disorders like osteogenesis imperfect and Ehler–Danlos. They also name a long list of factors as secondary causes of pediatric osteoporosis that include neuromuscular disorders (cerebral palsy and Duchenne muscular dystrophy), childhood cancer, endocrine disorders (Turner Syndrome and juvenile diabetes mellitus), and inborn errors of metabolism (Gaucher disease) and Chronic diseases like thalassemia. Anticonvulsants, glucocorticoids, and various forms of chemotherapy may adversely affect normal skeletal maturation (Bogunovic et al., 2009).

4.6.2 Problems with DXA in pediatric

Bone mass densitometry by dual X-ray absorptiometry (DEXA) of the lumbar spine and femoral neck is recommended as one of the most reliable and non-invasive technique for the assessment of bone mass (Hamidi et al., 2008). This method is very common around the world and many pediatric studies about bone densitometry and body composition have been published by using this method. (Van Kuijk, 2010).WHO osteoporosis diagnostic criteria should not be applied to children. We can't use T-score because children have not reached PBM, yet. Instead, in children, Z-score must be noticed, that it is a comparison of BMD of child to pediatric normative data. If the z- score is below -2 , we can use the term 'low bone density for chronologic age" (Daniels et al., 2003). DXA is reliable and accurate for adult but in children there is a challenge for it. As it is known, true bone density is a result of dividing BMC(g) by volume(cm3). In DXA , BMD is determined by dividing BMC by 2 dimensional area of a three dimensional objective (bone). By the use of these criteria smaller bone appear to have a lower BMD than larger bones. (Bogunovic et al., 2009). Bone size does not change, in adults, over time. On the contrary, bone size changes in growing children in 3 dimentions. When we screen children with DXA and follow them over time, we actually measure their growth instead of measuring actual changing in BMD.(Van Kuijk, 2010). It must be remembered that wide variation of height, and bone size in children makes interpretation of BMD difficult, especially in short children. Bogunovic et al., mention that longitudinal evaluation of a given patient over time is affected by the ever-changing size of the growing skeleton and the rates of skeletal growth vary with each bony dimension (Bogunovic et al., 2009). All this problems, cause to ask a question: Is it right to use DXA for measuring bone density and fracture risk in children or not? In response we emphasize some useful points about DXA. First it has fewer radiation than other methods, that is very important in radiology of children, 2) it is not a fearful (less noisy with no tunnel) method for children densitometry, 3) It is used worldwide and many pediatric studies, have been published in the field of bone densitometry and in the field of body composition studies, by using DXA method also 4) Studies about the relationship between bone density and fractures in healthy children, suggested that bone mass may contribute to fracture risk in childhood (Van Kuijk, 2010). So may be the answer is that performing DXA for measurement bone density and fracture risk in children, is a helpful method yet. However

we should emphasize that bone fragility in children extends beyond single BMD measurement, and bone geometry and body size influence it and in the diagnosis of osteoporosis, the presence of both a clinically significant fracture history and low bone mass, must be noticed (Bogunovic et al., 2009).

4.6.3 Special consideration of comparison of normal children and children with chronic disease, some points in BMD of chronic ill children

The measurement of BMC (g/cm) and BMD (g/cm2) are not only dependent on the mineral density of cortical and spongious bone, but also depend on the bone geometry. Lower BMD or BMC in shorter children may not describe a mineral deficiency or mineralization disorder, as is often thought, because the smaller bone may show lower BMD because of properties of DXA methods (Schonau,1998). BMD measurement in children is more affected by the wide variation of age at onset and progression of puberty. This leads to a wide variation in the age at reach of peak bone mass. It is thought the presence of a chronic disease, like juvenile arthritis, cause delay in pubertal onset and development. It has been estimated that one-third to one-half of the total mineralization in the lumbar spine in adult women is occurred during the 3 years around the onset of puberty. Therefore, we can t compare the BMD of a well-grown 13-year-old girl who is in mid-puberty with that of a small pre-pubertal 13-year-old with juvenile arthritis. Rabinovich remembers us that a DXA scan is not needed to tell who has the lower BMD. The question then is, is the BMD result in this small pre-pubertal girl normal? (Rabinovich, 2004).

As van Kuijk suggests, children with chronic disorders or medication, should never be compared with age-matched reference (normal) values. They should be compared with children with the same maturation status (skeletal age) (Van Kuijk, 2010).

5. Geometry (Another use of dual x-ray absorptiometry)

Some important factors such as the shape and structure of bone and the risk of falling, affect susceptibility to fracture so BMD alone cannot exactly predict who will have fracture. As Gregory and Aspden emphasize, the geometry of the proximal femur is a vital component in determining a person's risk of hip fracture. When a trauma occurs, such as a fall, the shape and structure of the femur determines how the forces are passed through the bone from the point of impact and whether they surpass the inherent strength of the bone and result in a fracture or not. Geometry component is seen in the picture from Gregory and Aspden article (Fig. 5.)

They explained any of these components

- Hip axis length: The distance from greater trochanter to inner pelvic brim, shown between points A and C in Fig. 5
- Femoral neck axis length (FNAL):

Femoral neck axis length is the linear distance measured from the base of the greater trochanter to the apex of the femoral head. It is illustrated by points B to C in Fig. 5. Confusingly, it is also sometimes referred to in the literature as hip axis length.

- Femoral neck width (FNW):

The narrowest distance across the femoral neck, often constrained to being perpendicular to the neck axis. The distance between points F and G in Fig. 5.

- Neck-shaft angle:

Usually defined as the angle between the femoral neck axis and the shaft axis (angle at point H in Fig. 5).

- Other geometrical measures: In addition to the most common measures of geometry discussed above, a number of other measures have also been related to fracture; including a thinner femoral shaft cortex , a thinner femoral neck cortex , a smaller calcar femoral (a dense, vertically orientated bone present in the posteroemedial region of the femoral shaft under the lesser trochanter of the femur) , a narrower trochanteric width and smaller inner and outer pelvic diameters. In contrast, an increased femoral head diameter has been related to increased bone strength.

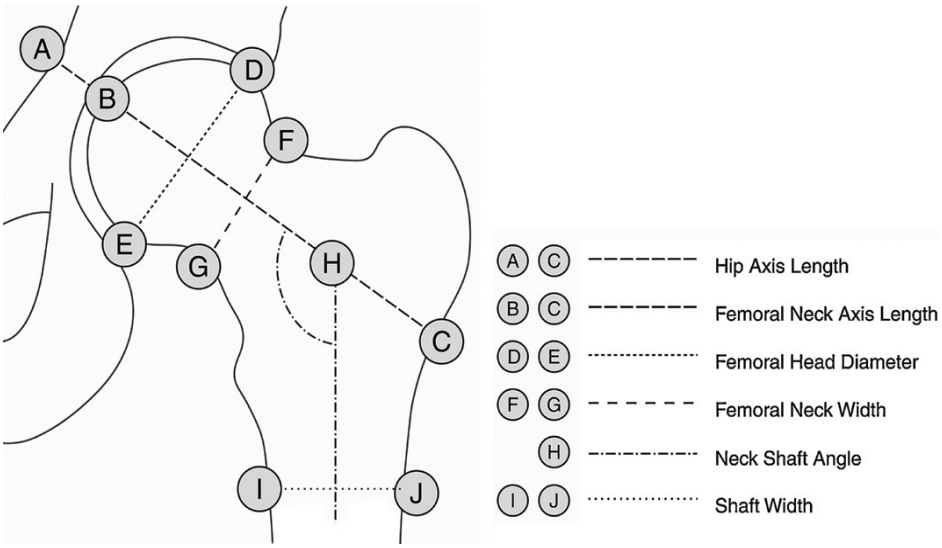

Fig. 5. Diagram illustrating some of the most common geometrical measurements made from the proximal femur (from Gregory and Aspden, 2008).

Two methods are must commonly used for assessing bone geometry, radiography and dual energy X-ray absorptiometry (DXA) (fan beam devices, more provide this service). Each of them; has its own advantages and disadvantages. Femoral geometry is important in determining both bone strength and fracture risk. The strongest associations with both outcomes appear to be a longer (Hip axis length) HAL and larger NSA (Neck-shaft angle) (Gregory & Aspden, 2008).

6. Finite element (An helpful method for better understanding of bone)

Need to a mathematical tool for solving complex mathematical problems, is answered by inventing Finite-element modeling (FEM). It helps to understand patterns of stress, strain, deflections, heat transfer, fluid flow, etc., in computer models of organic structures. Ross, emphasize that FEM provides a method for addressing a range of questions that are otherwise intractable, or very difficult to solve -in vivo or in vitro- and is potentially one of the most powerful tools in the methodological tool of vertebrate biomechanics. For example, clarifying functional consequences of the remarkable histological and morphological diversity of the vertebrae, is one of the important aims of vertebrate biomechanics. Many of researches on various disorders or diseases of the bone, are relied on this structure-function relationship. Skeletal health during long term space flight, as well as interpretation of skeletons found in the fossil and archeological records, are benefitted from these researches. Ross mentions that form-

function relationships of the skeleton are therefore of concern to bioengineers, clinicians, biological anthropologists, and paleontologists, and FEM provides a method for studying them. He also suggests that the availability of increasingly powerful computers at progressively more affordable prices has made FEM an accessible tool for biomechanists and the wide use of FEM in clinical research is now imitating many basic science researches (Ross, 2005). Finite element can be helpful in femoral characteristics finding as helpful as is in spinal vertebrae and finding the mechanisms and risk factors for fracture.

7. Recent progress in bone imaging for osteoporosis research

Development in bone imaging techniques have provided tools for analyzing bone structure at the macro-, micro- and nano-level. Ito, provided a list of recent progress in bone imaging as

- High-resolution CT (HR-CT) and high-resolution magnetic resonance (HR-MR). They are in vivo quantitative techniques for assessing the microstructure of trabecular bone non-invasively and non-destructively. Compared with MR imaging, CT-based techniques have the advantage of directly visualizing the bone in the axial skeleton, with high spatial resolution (of course, disadvantage of delivering a considerable radiation dose remains).
- Micro-CT (μCT) and Synchrotron μCT (SR-CT). The farmer provides a higher resolution of the microstructure and is principally applicable in vitro, has undergone technological advances such that it is now able to elucidate the physiological skeletal change mechanisms associated with aging and determine the effects of therapeutic intervention on the bone microstructure. In particular, synchrotron μCT (SR-CT) provides a more detailed view of trabecular structure at the nano-level.
- DXA-based hip structure analysis (HSA) and CT-based HAS. DXA-based HSA is a convenient tool for analyzing biomechanical properties and for assuming cross-sectional hip geometry based on two-dimensional (2D) data. CT-based HSA provides these parameters three-dimensionally in robust relationship with biomechanical properties, at the cost of greater radiation exposure and the lengthy time required for the analytical procedure.

The author, suggests that further progress in bone imaging technology is promising to bring new aspects of bone structure in relation to bone strength to light, and to establish a means for analyzing bone structural properties in the everyday clinical setting (Ito, 2011).

8. Conclusion

Tanner in his article reminds us the Bonnick suggestion (noted in the preface of the most recent edition of the author's book on bone densitometry in clinical practice)". . . as strange as it may seem, the technology itself is in danger of becoming so devalued that improvements in accessibility and advances in applications may be lost." (Bonnick SL. as cited in Tanner, 2011 from book "Bone densitometry in clinical practice: application and interpretation"(current clinical practice series). 3rd ed. Totowa, New Jersey:Humana Press; 2010). The future of DXA bone density testing is challenged by reimbursement, complicated guidelines, and the controversy over the monitoring of treatment. Nevertheless, bone health assessment and fracture risk prediction rely on quality bone density measurement using DXA (Tanner, 2011).

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Part 4

Secondary Osteoporosis

13

Patchy Osteoporosis in Complex Regional Pain Syndrome

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1. Introduction

Complex regional pain syndrome (CRPS), formerly known as "reflex sympathetic dystrophy" and "causalgia", is a syndrome that refers to a chronic pain condition associated with autonomic disturbances of vasomotor and sudomotor origin (Birklein et al., 1998), along with trophic skin changes and patchy demineralization of the bones (Poplawski et al., 1983). CRPS is classified into type I and II; the former can develop after minor or remote trauma like stroke, spinal cord injury or myocardial infarction (Wasner et al., 1998); the latter can develop after a large peripheral nerve lesion (Janig & Baron, 2003). The syndrome corresponding to what was formerly described as reflex sympathetic dystrophy is now termed as CRPS type I; causalgia is now termed as CRPS type II (Merseky & Bogduk, 1994). Although the mechanism of CRPS has not been elucidated yet, recent studies indicate that it is a complex disorder that involves both the central and peripheral nervous systems (Daemen et al., 1998; Huygen et al., 2001). CRPS pathogenesis is heterogeneous and complex, which makes its treatment challenging. Pharmacological therapies of CRPS include anti-inflammatory drugs, systemic corticosteroid (Kingery, 1997), antidepressants, opioid (Mackey & Feinberg, 2007), anticonvulsants, free-radical scavengers, vasodilatory medication (Perez et al., 2010) and even bisphosphonate agents (Adami et al., 1997; Manicourt et al., 2004; Robinson et al., 2004; Varenna et al., 2000). In addition, vitamin C is recommended to prevent the occurrence of CRPS type I after wrist fracture (Perez et al., 2010).

However, there is yet no single pharmacological agent or treatment algorithm that can resolve all of its heterogenic features. The efficacy for most pharmacological agents remains largely empirical, with the exception of bisphosphonate agents, which are the only agents with proven efficacy for CRPS based on multiple controlled trials (Adami et al., 1997; Brunner et al., 2009; Mackey & Feinberg, 2007;, Manicourt et al., 2004, Robinson et al., 2004, Varenna et al., 2000).

In order to understand how these bisphosphonate agents are useful in CRPS treatment, it is imperative to understand the pathogenesis of patchy osteoporosis in CRPS. This section will first review CRPS, then it will introduce the different experimental animal models. Finally this section will discuss the different treatment agents that have been studied for patchy osteoporosis.

2. Clinical findings of CRPS

2.1 Overview of CRPS

CRPS is painful and it can affect one or more extremities (de Mos et al., 2008). It usually occurs following a physical injury, such as, after fracture or surgery. But spontaneous onset without any triggering factor may occur as well (Veldman et al., 1993). According to a case control study (de Mos et al., 2008), fracture was the most common precipitating injury in 49% of the cases. The mixed etiologies of CRPS are evidenced in its heterogeneous constellation of clinical symptoms. In the acute stages, hallmarks include mechanical hyperalgesia, edema, increased sweating, skin temperature and hair growth (Doury, 1988; Janig & Baron, 2003). After some time, CRPS symptoms progress from a warm to a cold stage, with decrease of skin temperature, formation of skin atrophy and bony osteoporotic changes (van der Laan et al., 1998).

2.2 Diagnosis

CRPS diagnosis is based on its clinical presentation, whereby the diagnostic criteria as developed by the International Association for the Study of Pain (IASP) is most widely accepted (Stanton-Hicks et al., 1995). The IASP task force proposed a definition based on four criteria (Harden et al., 2007). (Table 1) In addition, involuntary movements, muscle spasm, paresis, pseudoparalysis, skin, muscle and bone atrophy, hyperhidrosis and changes in hair and nail growth, can also be observed (Perez et al., 2010; Veldman et al., 1993).

General definition of the syndrome: CRPS describes an array of painful conditions that are characterized by a continuing (spontaneous and/or evoked) regional pain that is seemingly disproportionate in time or degree to the usual course of any known trauma or other lesion. The pain is regional (not in a specific nerve territory or dermatome) and usually has a distal predominance of abnormal sensory, motor, sudomotor, vasomotor, and/or trophic findings. The syndrome shows variable progression over time
To make the clinical diagnosis, the following criteria must be met: 1. Continuing pain, which is disproportionate to any inciting event 2. Must report at least one symptom in three of the four following categories: Sensory: Reports of hyperesthesia and/or allodynia Vasomotor: Reports of temperature asymmetry and/or skin color changes and/or skin color asymmetry Sudomotor/Edema: Reports of edema and/or sweating changes and/or sweating asymmetry Motor/Trophic: Reports of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin) 3. Must display at least one sign at time of evaluation in two or more of the following categories: Sensory: Evidence of hyperalgesia (to pinprick) and/or allodynia (to light touch and/or temperature sensation and/or deep somatic pressure and/or joint movement) Vasomotor: Evidence of temperature asymmetry (>1°C) and/or skin color changes and/or asymmetry Sudomotor/Edema: Evidence of edema and/or sweating changes and/or sweating asymmetry Motor/Trophic: Evidence of decreased range of motion and/or motor dysfunction (weakness, tremor, dystonia) and/or trophic changes (hair, nail, skin) **4.** There is no other diagnosis that better explains the signs and symptoms
For research purposes, as a rule, CRPS is diagnosed when least one of the symptoms in all of the four symptom categories and at least one sign (observed at evaluation) in two or more sign categories is manifested.

Table 1. Proposed clinical diagnostic criteria for CRPS

Plain radiographs can be used to evaluate the demineralization status, but these show positive findings only in the chronic stages. Three-phase bone scintigraphy is a highly specific and sensitive test for CRPS (Demangeat et al., 1998). The classical finding on bone scintigraphy is increased periarticular activity in the affected limb (Todorovic-Tirnamic et al., 1995). Autonomic function can be tested by infrared thermography. Also, skin temperature differences may be helpful for the diagnosis of CRPS; however, these typical temperature side differences are not static descriptors but comprise changes that can be critically dependent on environmental temperature (Wasner et al., 2001).

2.3 Pathomechanism

The complex cascade of CRPS is postulated to initiate after the sensitization of C-nociceptive fibers and release of neuropeptides, which are linked to vasodilatation and hypersensitization of nerve endings (Guo et al., 2004; Kurvers, 1998; Schurmann et al., 1999). Osteoclasts are also activated and this in turn leads to nociceptor stimulation and sensitization (Mach et al., 2002; Sevcik et al., 2004) leading to a vicious cycle.

A medical history of asthma, migraine, osteoporosis, a recent history of menstrual cycle-related problems or preexisting neuropathies are common pre-existing problems or conditions often concomitantly found in CRPS patients. Therefore finding a common mediator that is both present in these conditions and CRPS could help to reveal the possible triggering factors. The mediators (de Mos et al., 2008; Karacan et al., 2004; Toda et al., 2006) that have been linked among asthma, migraine and CPRS are the neuropeptides calcitonin-gene related peptide, substance P (de Mos et al., 2008), mast cell products (Bradding et al., 2006) and transcription factors such as nuclear factor kappa B (Barnes, 2006; Reuter et al., 2002). Inflammatory cytokines such as interleukin 1, tumor necrosis factor alpha have also been suggested to be common denominators among CRPS, osteporosis and menstrual cycle related disorders, but their definite roles need to be established through continuous studies (Marie et al., 1993; Zarrabeitia et al., 1991).

3. Patchy osteoporosis

3.1 Pathomechanism

Why does bone loss occur in CRPS? Some have postulated that immobilization plays a role in CRPS. Suyama et al, have (Suyama et al., 2002) observed a reduction in BMD 1 to 7 weeks postsurgery with an increase in the number of osteoclasts at 2, 3, and 5 weeks in their CRPS model. They have suggested that one possible mechanism would be the increase of bone resorption with immobilization. Another possible mechanism suggested by others (Whiteside et al., 2006) would be that bone loss in CRPS models may be due to altered nerve signaling and not attributable to limb disuse or reduced mechanical loading associated with pain. Experimental studies have shown that substance P release is involved in the pathogenesis of bony changes induced by CRPS (Gaus et al., 2003).

The exact pathological mechanism of patchy osteoporosis in CRPS and altered nerve signaling is still poorly understood, some consider to be attributable to a regional sympathetic hyperactivity of sympathetic dysfunction (Goldstein et al., 2000; He et al., 2011; Kurvers et al., 1998; Laroche et al., 1997). Sympathetic deregulation causes vasomotor irregularities, and an imbalance between vasoconstriction and vasodilatation, which in turn influences the blood supply to the bone. Other studies have shown that the immune and skeletal systems are closely related to maintain the homeostasis of the bone but when this

interaction is disrupted in CRPS; the balance favors bone loss. This complex cascade is postulated to initiate after sensitization of C-nociceptive fibers and release of neuropeptides, which are linked to vasodilatation, hypersensitization of nerve endings (Guo et al., 2004; Kurvers et al., 1995; Schurmann et al., 1999), and osteoclasts activation, which increase bone resorption, lead to nociceptor stimulation and sensitization (Mach et al., 2002; Sevcik et al., 2004).

3.2 Characteristics

Bone loss in CRPS occurs regionally with loss of the trabecular bone (Bickerstaff et al., 1991; Doury, 1988) with marked bone demineralization observed at the subchondral regions. Epiphyseal regions are predominantly affected, however no narrowing of joint space or bony sclerosis is observed. Recovery of lost bone mineral content is slow and may persist after several years from the initial diagnosis (Nilsson, 1966) and this persistent regional osteoporosis can predispose to other future fractures after minor injuries (Sarangi et al., 1993). Same as the clinical manifestation, studies that have used rat models of CRPS have shown that bone mineral density significantly decrease from the second week (Suyama et al., 2002) and this loss is known to persist for at least 20 weeks (Kingery et al., 2003).

3.3 Radiographic findings

Bone changes can be observed by typical roentgenography but these changes are known to occur only after several months. However periarticular bone loss can be observed in radiograhs of CRPS limbs even within 3 weeks after injury (Bickerstaff et al., 1993). Bone mineral density, measured by dual energy xray abosorpotometry is reduced in the CRPS limbs in a periarticular distribution (Gue et al., 2004).

3.4 Neuropeptides in patchy osteoporosis

Substance P (Bianchi et al., 2008), one of the neuropeptides closely linked to the pathogenesis of CRPS, binds to NK1 receptors of postcapillary venules and causes vasodilation, increasing vascular permeability. The increased activity of this neuropeptide, which are elevated in serum samples from CRPS patients (Schinkel et al., 2006), are deemed to be responsible for the subsequent warmth and interstitial edema observed in CRPS through vasodilation and increased protein extravasation.

This substance P is also postulated to play a role in the development of patchy osteoporosis in CRPS. Studies have shown that substance P is known to stimulate osteoclast formation and active bone resorption through NK1-receptor found in the bone cells (Goto et al., 1998; Liu et al., 2007).

The exact mechanism of how substance P induces bone loss needs to be elucidated; substance P not only has osteoclastic effects but is known to have an osteogenic effect on bone marrow cells and to directly stimulate osteoblastic bone formation (Imai & Matsusue, 2002). The mechanism that favors osteoclastic activation, instead of osteoblastic activation, to result in bone loss in CRPS needs further studies. But in line with the current literature that supports abnormal osteoclastic activation through substance P in CRPS, it is reasonable to theorize that an agent that inhibits substance P would help to reduce osteoclast activation and its ensuing bone loss. This topic was evaluated in a study that used a substance P antagonist LY303870 (Kingery et al., 2003) and determined whether it was efficient in controlling osteoporosis. Although this antagonist was effective in the nociceptive and

vascular abnormalities (Kingery et al., 2003), it proved to be ineffective in preserving bone loss. Its use instead enhanced the widespread osteoporotic effects (Kingery et al., 2003). The dual and dichotomous roles of substance P in maintaining bone integrity in CRPS needs to be further elucidated.

Substance P activation also leads to the over-expression of the inflammatory cytokines (Wei et al., 2009). Some of these cytokines play a role in the development of patchy osteoporosis in CRPS. Nerve growth factor is one of the cytokines activated by the substance P, and its activity leads to nociceptive sensitization, enhanced osteopenia with increased cytokine content (Sabsovich et al., 2008). Tumor necrosis factor alpha is another pro-inflammatory cytokine postulated to play a role in the development of CRPS changes after trauma and its expression is increased in CRPS patients (Huygen et al., 2001). Although the increased level of the tumor necrosis factor is an important mediator of regional nociceptive sensitization, it does not contribute to the enhanced bone loss (Sabsovich et al., 2008).

4. Current laboratory research in osteoporosis related with CRPS

4.1 Overview of laboratory research in CRPS

In order to unravel the mechanisms underlying osteoporosis in CRPS, many animal models have been introduced. There are two broad categories of mechanisms underlying CRPS: (1) peripheral mechanisms: CRPS is primarily an inflammatory disease in the periphery (CRPS I) or a consequence of nerve damage (CRPS II), (2) central mechanisms that involve reorganization of the somatosensory, somatomotor and autonomic systems in the central nervous system triggered by a peripheral input (Drummond et al., 2001; Turner-Stokes, 2002; Wasner et al., 2003). Both the peripheral and central nervous systems play a role in the pathogenesis of CRPS. The peripheral mechanisms includes immune cell mediated inflammatory, autoimmune inflammatory processes, neurogenic inflammation and tissue hypoxia. (Daemen et al., 1998a; Daemen et al., 1998b; Kingery et al., 2003b; Kurvers et al., 1998; Offley et al., 2005; Schurmann et al., 2000). However, the amount of contribution of these two mechanisms and how they interact with each other to manifest in CPRS has not been determined yet. Keeping in mind of these two different mechanisms, and that CRPS can be either type I or II, several animal models that represent these features have been introduced but because of the inherent heterogenic features of CRPS, there is no absolute model that shows and reproduces all CRPS features.

Depending on the presence of peripheral nerve injury, three animal models will be discussed. For CRPS type I, the tibia fracture model and chronic ischemic model will be presented (Coderre et al., 2004; Guo et al., 2006; Ludwig et al., 2007). The chronic constriction injury (CCI) model of the sciatic nerve will be presented for CRPS type II (Bennett and Xie, 1988). Choosing the type of experimental model may depend on the objective of the research or researcher's habit. The methodologies of these different animal models are discussed to provide detail reference for the readers.

4.2 Tibia fracture and cast rat model

Tibia fracture and cast rat model had been introduced for the animal model of CRPS type I, and is popularly used in laboratory studies (Guo et al., 2004; Guo et al., 2006; Sarangi et al., 1993). The method of induction for tibia fracture model is as follows: the hind limb of rat is wrapped in stockinet and the distal tibia is fractured. The hind limb is then wrapped in casting tape with the hip, knee, and ankle in flexed position. The cast extends from the

metatarsals of the hindpaw up to a spica formed around the abdomen. At 4 weeks the cast is removed. This rat model shows changes in volume, temperature, nociception and osteoporosis of the hind limb.
This tibia fracture and cast model has several benefits. Most of all, this animal model represents the CRPS type 1. This model is theorized to induce post-junctional facilitation of substance P signaling. Because this model reproduces the typical symptoms of CRPS such as mechanical allodynia, paw thickness (edema), vasodilation and bone mineralization, it is commonly used in research studies that focus on the treatment and pathomechanism of CRPS. The exact mechanisms of how the intact peptidergic primary afferent neurons are activated after fracture and casting has not elucidated yet. Although there are many studies that have used this model to investigate the pathomechanism of CRPS type I, there are yet no studies that have exclusively focused on patchy osteoporosis with this model.

4.3 Ischemic – Reperfusion injury model

Another typical animal model for CRPS type I is the chronic ischemic model (Coderre et al., 2004; Xanthos et al., 2004). The femoral artery is dissected and ligated above the origin of the profunda femoris artery for the 3 hours with a small polyethylene tube. Ligation is performed tightly with the vessel walls pressed together and complete arterial occlusion is ensured under microscope. This method completely interrupts the arterial blood supply to the lower leg and hindpaw. The wound is closed by means of five sutures put on the skin. To prevent thrombosis of the artery, two subcutaneous injections of heparin are given subcutaneously, one at the beginning and one at the end of the period of ischemia. This ischemic injury shows change of skin temperature, spontaneous pain behavior, mechanical and cold allodynia and edema, and are consistent with CRPS type I.
Previous research revealed that CRPS type I may depend on chronic tissue ischemia that is dependent on, or exacerbated by, an indirect sympathetic–afferent coupling with an intervening role of enhanced a-adrenoceptor mediated vasoconstriction. The ischemia-reperfusion injury model is another animal model for CRPS type I that is produced based on this mechanism.

4.4 Chronic constriction injury (CCI) model

The CCI model; first introduced by Bennett and Xie (1998); is a classic model for CRPS and has been commonly used in various studies. In this model the common sciatic nerve is exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris. Proximal to the sciatic's trifurcation, about 7 mm of nerve is freed from adhering tissue and 4 ligatures are loosely tied loosely around it with 1 mm spacing. The length of the ligated nerve is approximately 4-5 mm long.
This model is known to represent CRPS type II. This model shows changes of skin thickness, temperature, mechanical sensitivity and bony changes such as patchy osteoporosis. This model has been the model most frequently used to study the patchy osteoporosis in CRPS. Patchy osteoporosis resembling that of CRPS can also be induced by the sciatic nerve trans-section (Kingery et al., 2003a; Kingery et al., 2003b). This model is also used for studies on CRPS type II. However, the CCI model had shown several benefits for weight bearing than the sciatic nerve trans-section model. Many of the laboratory researches on patchy osteoporosis in CRPS are mostly based on the CCI model.

5. Treatment of patchy osteoporosis in CRPS

5.1 Overview of medication for patchy osteoporosis

Pharmacological therapy of CRPS encompasses a wide spectrum of medication; from anti-inflammatory drugs, systemic corticosteroid (Kingery, 1997), antidepressants, opioid (Mackey & Feinberg, 2007), to anticonvulsants agents. Because the activation of bony osteoclasts is known to play significant role in CRPS pain generation, it is not surprising that aside from these central pain modulating medications, bone modulating agents are used in CRPS. These agents are known not only to alleviate pain but also to reverse and inhibit CRPS associated osteopenia (Whiteside et al., 2006). The two bone modulating agents in reference are calcitonin and bisphosphonate agents.

5.2 Calcitonin

Calcitonin has been traditionally used in bone pathologic conditions due to its efficacy on microvasculature, bone resorption and analgesic action (Friedman & Raisz, 1965). The use of calcitonin in CRPS has been shown through its possible mechanism in controlling bone pain. The results of calcitonin in clinical practice are still controversial; while some have questioned the efficacy (Kingery, 1997), others support its efficacy in CRPS pain (Perez et al., 2001). A recent review analysis also describes positive results for calcium-regulating drugs, including calcitonin, administered to CRPS patients (Fofouzanfar et al., 2002). Although calcitonin has some efficacy in pain, range of motion, with a rapid onset of action (Gobelet et al., 1992), whether its use has effect on the patchy osteoporosis in CRPS has not been validated through animal or clinical studies.

5.3 Bisphosphonate

5.3.1 Mechanism of bisphosphonate through experimental studies

Bisphosphonates are analogues of inorganic pyrophosphates and are inhibitors of bone resorption. They act on the bone and inhibit the action of osteoclasts, thereby limiting bone resorption. Due to this mechanism, they have been found to be effective in the treatment of osteoporosis and other bone conditions. Bisphosphonates have been used traditionally for pathological bone conditions, such as osteoporosis, Paget's disease, cancer related bone pain, metastatic cancer, tumor related hypercalcemia, myeloma and vertebral fracture (Adami et al., 1997; Brunner et al., 2009; Bonabello et al., 2001; Fleisch, 1997; Fulfaro et al., 1998; Fulfaro et al., 2005). In CRPS, bisphosphonates have shown more promising results than calcitonin and many studies supports itse use in CRPS (Adami et al., 1997; Breuer et al., 2008; Manicourt et al., 2004; Robinson et al., 2004; Varenna et al., 2000), in fact, bisphosphonates are the only pharmacological agents with beneficial analgesic results confirmed through placebo controlled trials (Adami et al., 1997; Manicourt et al., 2004; Varenna et al., 2000). However, there is yet no consensus on the optimum dosage, frequency, and duration of treatment in CPRS.

The role of bisphosphonate in the regulation of the substance P and hyperalgesia has been shown in an experimental study using ibandronate (Bianchi et al., 2008), a bisphosphonate agent. As stated earlier, substance P sensitizes afferent fibers and increases the sensitivity to nociceptive stimuli. It has been hypothesized that ibandronate prevents proton production by osteoclasts, and reduce the activation of specific ion channels and consequent production of substance P by primary afferents (Bianchi et al., 2008), thereby limiting hyperalgesia and bone loss. Also bony calcium homeostasis can influence the Ca^{2+} dependent endogenous regulation

of pain sensitivity (Bonabello et al., 2001). Bisphosphonates can effect bone tissue by alternation of the calcium/phosphate product. It is postulated that it is through these mechanisms that bisphosphonate administration inhibits the release of neuropeptides that are responsible for the pain and other vasomotor changes in CRPS. Also, it is postulated that it is through these same mechanisms that bisphosphonate agents are useful in limiting bone loss.
In fact, animal studies have shown bisphosphonates are effective in preserving CRPS associated bone loss. Chronic administration of zoledronate acid can lead to increased BMD in CCI animal models (Whiteside et al., 2006). The efficacy of alendronate in limiting bone loss in CCI rat model has been shown in a recent study (Im et al., 2010). In both the acute and chronic stages after CCI induction, alendronate treatment preserved bone mass with sustained efficacy in bone preservation, which was demonstrated through in vitro tibia BMD and tibia strength results.

5.3.2 Clinical studies of bisphosphonate

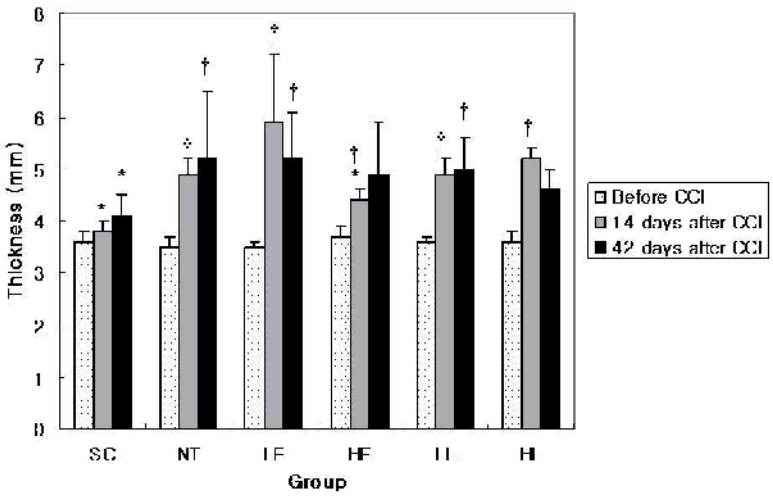
The role of bisphosphonates in CRPS is well supported by many clinical studies but most were focused on their efficacies in pain. There are already many reports that have advocated the use of bisphosphate agents for CRPS related hyperalgesia and pain (Adami et al., 1997; Breuer et al., 2008; Mackey & Feinberg, 2007; Manicourt et al., 2004; Robinson et al., 2004; Varenna et al., 2000). Results from clinical studies have postulated that alendroate reduces local bone resorption and is effective in CRPS pain by its nociceptive effects in bone. (Adami et al., 1997; Manicourt et al., 2004). For example, Varenna et al. have shown in their randomized, double blind placebo controlled study that a 10 day intravenous clondronate course is effective in the treatment of CRPS (Varenna et al., 2000). A recent clinical study of ibandronate, a potent bisphosphonate agent, has shown that its analgesic effects (Bianchi et al., 2008). However, most studies focused on their analgesic effects for bone pain rather than on their bone preserving effects.
Bone loss in CRPS predominate the chronic stages of CRPS and is accompanied by trophic changes. This patchy bone loss is difficult to reverse and as stated earlier, can lead to fractures even from trivial stress. Therefore, alongside with the management of pain, management of CRPS associated patchy osteoporosis is important to prevent such detrimental consequences.
The efficacy of bisphosphonate agents in patchy osteoporosis have been shown in some studies. A theraupetic role of bisphosphonates on clinical and densiometric recovery was shown in transient hip osteoporosis; a condition considered by many to be a prestage of CRPS (Mailis et al., 1992) with similar features commonly observed in CRPS. Administration of bisphosphonate in transient hip osteoporosis led to the recovery of bone densiometry along with complete pain resolution (Varenna et al., 1996). Similary, the efficacy of bisphosphonate therapy in the recovery of bone mineral content was also shown in CRPS (Adami et al., 1997). Adami et al. used intravenous alendronate and evaluated their pain, tenderness, swelling and bone mineral content of the affected arm. Although a change of bone mineral content was not observed in the unaffected side, the affected side bone mineral content rose significantly in comparison to baseline values. These results show that bisphosphonates are helpful in limiting CRPS associated pathcy osteoporosis.

5.4 Dosage and administration of bisphosphonate in patchy osteoporosis

With bisphosphonates as the agent with much clinical and experimental evidence to support its use in CRPS, the best dosage and timing of administration is an issue that has gained much focus. A study of dosage differentiation was previoulsy carried out with doses of pamidronate

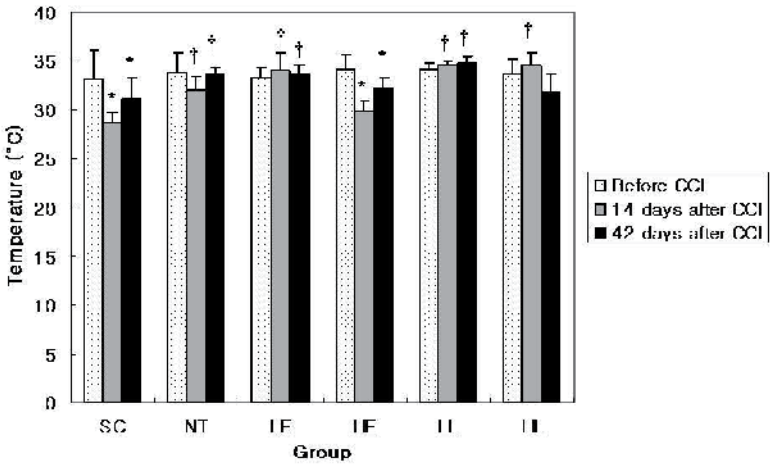
varying from 30mg/day to 1mg/Kg/day, provided for three consecutive days. However no dose correlation was observed in these clinical trials (Maillefert et al., 1995). In contrast, some animal studies have shown a dose dependent antinociceptive effects (Bonabello et al., 2001)with pamidronate and clodronate. Etidronate and alendronate have not shown this dose dependent response and their analgesic effects were observed only with the highest dose.

Similar to these experiments, in their study with CCI models, Im et al. has shown that different dosage and time of administration of oral alendronate leads to different results in bone mineral density of the tibia and tibia bone strength (Im et al., 2010). The high dosage group received 1mg/kg/day while the low dosage group received 0.1mg/kg/day. To determine whether the time of administration lead to significant differences, the high and low dosage groups were further divided into the early and late administered group. The early group received alendronate treatment immediately after CCI induction, while the late group received alendronate at the 14th day. Both groups received alendronate treatment until the 6th week of CCI induction. The results showed that different dosages and time of administration leads to different efficacies across different CRPS signs. While the hind paw thickness and temperature were significantly reduced only with high dosage administered immediately after CCI induction (Fig. 1, Fig. 2), bone strength and bone mineral density was significantly increased in the high dosage group, with both in the early and late admistered group (Fig. 3, Fig. 4). Bone loss in CRPS becomes manifest in the chronic stages and is known to progress over several months. Because bone loss predominates the later course of CRPS, the authors suggested that the high dosage alendronate, whether admnistred in the early or late course of CRPS, can show significant efficacy in bone metabolism.



*P<0.001 as compared with NT group, †P<0.001 as compared with SC group.
Abbreviations: CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment

Fig. 1. Efficacy of oral alendronate in different dosage and time of administration in dorsal-ventral thicknesses of the affected hind-paw from Sprague-Dawley rats. (Adapted from Im, S., Lim, S.H., Lee, J.I., Ko, Y.J., Park, J.H., Hong, B.Y. & Park, G.Y. Effective dosage and administration schedule of oral alendronate for non-nociceptive symptoms in rats with chronic constriction injury. Journal of Korean Medical Science 2010; 25(6): 938-944)



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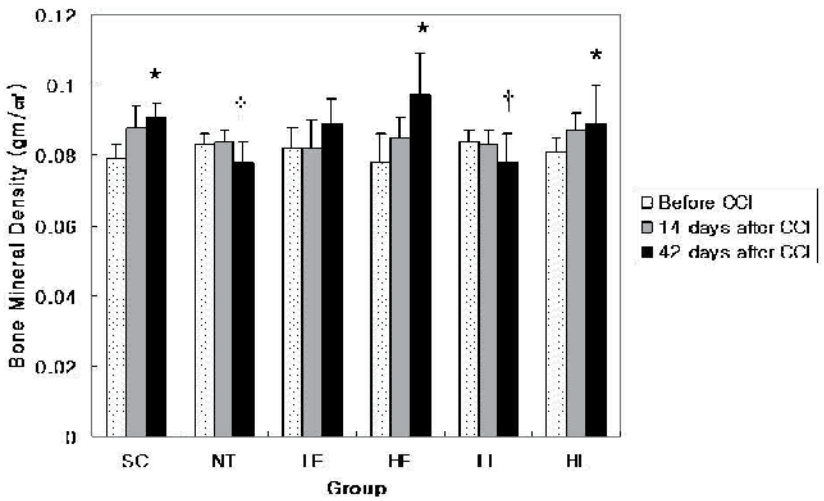
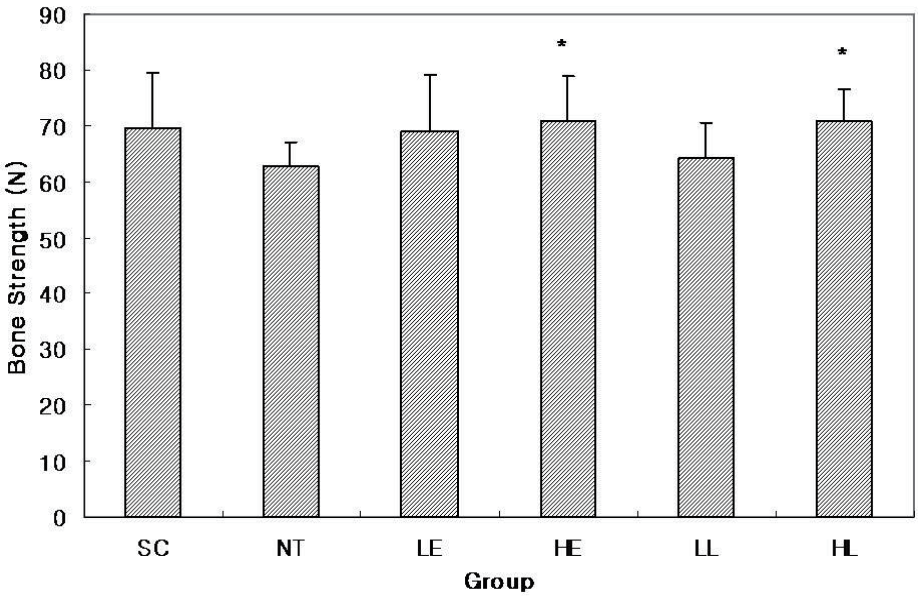
Abbreviations: CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment

Fig. 2. Efficacy of oral alendronate in different dosage and time of administration in skin temperature of the affected hind-paw from Sprague-Dawley rats. (Adapted from Im, S., Lim, S.H., Lee, J.I., Ko, Y.J., Park, J.H., Hong, B.Y. & Park, G.Y. Effective dosage and administration schedule of oral alendronate for non-nociceptive symptoms in rats with chronic constriction injury. Journal of Korean Medical Science 2010; 25(6): 938-944)



*P<0.001 as compared with NT group, †P<0.001 as compared with SC group.
Abbreviations: CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment

Fig. 3. Efficacy of oral alendronate in different dosage and time of administration in BMD of the affected tibia from Sprague-Dawley rats. (Adapted from Im, S., Lim, S.H., Lee, J.I., Ko, Y.J., Park, J.H., Hong, B.Y. & Park, G.Y. Effective dosage and administration schedule of oral alendronate for non-nociceptive symptoms in rats with chronic constriction injury. Journal of Korean Medical Science 2010; 25(6): 938-944)



*P<0.001 as compared with NT group
†P<0.001 as compared with SC group.
Abbreviations: CCI, chronic constriction injury; SC, sham control; NT, no treatment; LE, low dosage early treatment; HE, high dosage early treatment; LL, low dosage late treatment; HL, high dosage late treatment

Fig. 4. Efficacy of oral alendronate in different dosage and time of administration in bone strength of the right tibia from Sprague-Dawley rats, obtained after the rats were sacrificed. (Adapted from Im, S., Lim, S.H., Lee, J.I., Ko, Y.J., Park, J.H., Hong, B.Y. & Park, G.Y. Effective dosage and administration schedule of oral alendronate for non-nociceptive symptoms in rats with chronic constriction injury. Journal of Korean Medical Science 2010; 25(6): 938-944)

Despite these results, studies that evaluate on the appropriate human dosage of bisphosphonates to alleviate CRPS associated bone loss are warranted in future studies. Although previous studies have shown variable efficacy of bisphosphonate agents with different dosages and time of administration for the different signs of CRPS, the dosage administered in the high dosage group was approximately 5–6 times higher than standard clinical dosages, thus, the high dosage used in experimental studies poses potential problems to directly administer to humans.

Responses to bisphosphonates can vary depending on which agent is used and also on when and how these agents are administered. More experimental studies that asses the efficacy of different bisphosphonate dosages and time of administration in pain and temperature are needed to translate these findings to clinical usage. Also, it would be of interest to determine if prophylactic high dosage bisphosphonate administration in CRPS are helpful in limiting the bone loss that continues until the later stages of CRPS. Finally long term follow-up clinical data are needed to evaluate the efficacy of bisphosphonates in limiting bone loss through objective evidence from bone densiometry and bone markers.

5.5 Neuropeptide modulators

Because the pathogenesis of patchy osteoporosis is related to neurogenic inflammation and the production of substance P, many studies that targeted these neuropeptides have been published. As stated earlier, substance P activation also leads to the over-expression of the inflammatory cytokines (Wei et al., 2009), for example, nerve growth factor is one of the cytokines that leads to osteopenia. The use of a nerve growth factor antibody not only reduced nociception but to a modest degree, maintained further bone loss in the distal trabecular bone (Sabsovich et al., 2008). Pentoxifylline, a cytokine inhibitor, was used to evalute its effect in trabecular bone loss (Wei et al., 2009). Pentoxifylline had significant effects in the fracture induced up-regulation of inflammatory cytokines and reversed nociceptive sensitization and vascular abnormalities. However, it had insignificant effects on bone architecture as measured by microcomputed tomography in a tibia fracture model of CRPS. Although pentoxifylline treatment can induce osteoblastic differentiation, it had no significant effect on trabecular bone loss (Sabsovich et al., 2008).

Although the exact mechanism and relationship of osteoclastic activation, with subsequent activation of substance P and inflammatory cytokines needs further evaluation, most experimental studies have shown that only the agents that directly inhibit bone resorption through osteoclast inhibition have efficacy in preserving CRPS associated bone loss. To date, bisphosphonate agents are ideal for controlling the pain and for limiting bone loss in CRPS.

6. Conclusion

The main focus in CRPS both in clinical and experimental settings has been focused on hyperalgesia and vasomotor symptoms. The symptoms are manifest from the early stages of disease, are profound and affects patients' quality of life. In contrast, patchy osteoporosis in CRPS are not apparent until the later stages, and bone loss rarely causes any symptoms until a minor trauma leads to unexpected fractures. Despite the different clincal manifestations of hyperalgesia and osteoporosis, and the tendency to divide CRPS into different stages, all the signs of CRPS are in continuum and dependent on one another; one sign of CRPS does not stand alone and one can not exist without the other, therefore simply aiming the treatment focused on one aspect can not limit the heterogenic features of CRPS. Vasomotor and sudomotor signs and patchy osteoporosis in CRPS are triggered through similar pathways and neuropeptides are the mediators that link them together. To date, bisphosphonates in high dosages have been used with the aim to control these neuropeptides through osteoclastic modulation. Future studies and clinical trials are warranted for the treatment of CRPS patchy osteoporosis.

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14

Osteoporosis in Microgravity Environments

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1. Introduction

All life on earth has evolved in, and via the adaption to, the presence of gravity. This includes humans who branched off from a distant ancestor about five to seven million years ago. On April 12, 1961, one such human---Yuri Gagarin of the Soviet Union---took off in Vostok 3KA for the first trip into outer space. Since then, numerous trips to the moon, the Skylab, and within the Space Shuttle have followed. With the recent completion of the International Space Station, the current focus is set on very long duration crewed missions to the station, the establishment of a potential lunar outpost, and possible exploration of Mars.

As more and more humans head to space for longer and longer periods of time---out of desire or necessity---significant challenges will be faced. The *technological* challenges will undoubtedly be met. The past fifty years have taught us that given adequate time and financial resources nearly any technological hurdle can be jumped. The *biological* challenges are far greater. As noted, all human life on earth has evolved via adaption to gravity and long-term exposure to microgravity takes its toll; especially on the musculoskeletal, cardiovascular, sensory-motor, and immune systems.

In this chapter, we will review the known effects of long-term microgravity on the skeletal system, examine what is as-yet unknown, and explore possible interventions that might be used to address these effects.

2. The impact of microgravity at the cellular level

2.1 Osteoporosis on earth

Osteoporosis occurring on earth in the presence of normal gravity is most often associated with aging and most significantly impacted by peak bone mass and the rate of bone loss thereafter. Peak bone mass is generally achieved while humans are in their early thirties and subsequent bone loss is impacted not only by aging and menopause (women), but by hereditary predispositions, exogenous factors (such as alcohol, smoking, inactivity, malnutrition, prescription medications, etc.), and disease states (such as endocrine disorders, renal disorders, rheumatologic disorders, etc.). Each of these causes results in a final common pathway leading to osteoporosis---an imbalance between bone formation and bone resorption. Fractures, primarily of the proximal femur ('hip'), vertebral bodies, and distal radius ('wrist') are significant risks and, as other chapters in this text have outlined, represent important causes of morbidity and potential mortality.

Osteoblast and osteoclast uncoupling is the primary source of this excessive resorption and biomechanical fragility of bone. If the cause can be determined, then reasonable solutions aimed at such uncoupling can be offered to address the problem. Bispohosphonates and, more primitively phosphate, can impede osteoclastic resorption. Calcium, Vitamin D, calcitonin, estrogen, exercise, smoking and alcohol restriction, and avoidance of particular medications can help halt bone loss. Fluoride (no longer used), parathytoid hormone, and aggressive exercise might result in bone mass gain.

2.2 Osteoporosis in microgravity

Osteoporosis occurring as a result of microgravity is, from the perspective of the organism down to the lowest biological level, *different* than that encountered on earth.

2.2.1 Cytoskeletal alterations

Microgravity appears to significantly alter the cellular cytoskeleton. Proper cytoskeletal structure allows intracellular proteins to participate in important functions such as mitosis, cell motility, intracellular transport, and organization of organelles. Actin filaments, intermediate filaments, and microtubules are the key elements and they serve as a highly organized dynamic scaffold on which intracellular processes take place.

In microgravity, cellular structure, intracellular organization, and micro-fluid dynamics are altered[1]. Disruption of normal biochemical and physiological processes follows. Clement and Slenzka[2] have demonstrated that the spatial relationships between cellular organelles and structures are abnormal. And He[3] and Crawford-Young[4] have demonstrated that cellular cytoskeletal and microfilament dynamics are anomalous and might well be the source. Thus DNA replication, RNA transcription, protein migration, and ionic and molecular transport are perturbed.

2.2.2 Mesenchymal stem cells

The impact of these intracellular changes is felt by mesenchymal stem cells (MSC). MSC---present in adult life in the periosteum of bones and within the bone marrow---differentiate into osteoblasts following appropriate signaling and presence within the proper mileau. Meyers[5], Yuge[6], Huang[7], and Pan[8] (in separate studies) have demonstrated via flow cytometry, transcriptional analyses, and proteomic analyses that MSCs ability to proliferate, to differentiate into osteoblasts, and to contribute to osteogenesis is inhibited by microgravity.

2.2.3 Osteoblasts

Osteoblasts are also directly compromised. Bucaro[9] has demonstrated findings that suggest that direct induction of osteoblast apoptosis occurs in microgravity. Apoptosis is differentiated from usual cell necrosis (where cells swell, burst and die) by characteristic intracellular changes including nuclear condensation and shrinkage and cytoplasmic vacuolization. Observed osteoblastic apoptosis likely results from cytoskeletal changes.

Additionally, Colleran[10] has noted that the cephallic fluid shift experienced by humans in microgravity might alter interstitial fluid pressures and flows and, given that osteoblasts survive somewhat tenuously in low flow areas, these shifts might result in cell functional compromise or death.

3. The impact of microgravity at the systemic level

Systemically, microgravity induces osteoporosis via the above noted unique cellular changes *coupled* with an environment of nearly non-existent mechanical stresses where normal weight-bearing and the normal response of bone to proliferate accordingly (Wolff's Law) is altered. And this alteration differs than, say, that seen with immobilization. While patients placed in body casts and on bed rest (fully non-weightbearing) will suffer from osteoporortic changes, the amount of calcified bony tissue lost over three months is generally about 3%, tends to then level off at about three months (no further loss), and tends to be reversed with resumption of weight-bearing. In microgravity, the loss occurs at four times the rate, does not appear to level off, and appears to be much less reversible. Thus, the one-year trip to Mars is estimated to potentially result in a (devastating) greater than 25% reduction of bone mass. And this is in astronauts; predominantly male, at an age where their bone mass is at peak levels, exposed to no exogenous factors (smoking, excessive alcohol, etc.), in prime physical condition, and with no underlying disease states.

Simply, the combination of altered cellular form and function coupled with differences in bony response to microgravity systemically means that this form of osteoporosis bears relatively little relation to that seen on earth and that astronauts experience early, aggressive, continual bone loss. Predictably, systemic markers of bone resorption are greatly increased, while markers of bone formation are decreased[11] to levels rarely seen in on-earth conditions. And, importantly, it is unclear whether these changes are fully reversible upon return to earth and 'normal' gravity conditions.

4. Bone health and present day human spaceflight

Since the earliest days of human spaceflight, physiologists and NASA flight surgeons recognized the importance of exercise to maintain musculoskeletal and cardiovascular health. Owing to prolonged exposure to microgravity, Astronaut crews returning from America's first space station, Skylab, were too weak to stand upon return to earth. Exercise equipment thus became a requirement for all long duration space missions. A series of devices, including treadmills, stationary bicycles, rowing ergometers, simple resistive exercise systems and complex, reconfigurable "weight machines" have evolved in the years since, both in the Soviet-turned-Russian space program and now in the US-led International Space Station (ISS) program.

Exercise devices designed to maintain cardiovascular fitness in the absence of gravity proved to be a more straightforward engineering goal: movement against a friction wheel can easily challenge the cardiopulmonary system. Providing resistive exercise challenge to the postural musculoskeletal system of sufficient intensity and quality has only recently been accomplished aboard the ISS. The Advanced Resistive Exercise Device (ARED) uses pistons to provide smooth exercise loads, and is highly reconfigurable for a wide array of concentric and eccentric exercises.

The world record duration in space is held by Dr. Valeri Polyakov, who spent 437 consecutive days in microgravity, landing in 1995. During his endurance mission he was required to exercise up to four hours a day. Human spaceflight is very costly, but is obviously undertaken to accomplish important scientific goals in life sciences, material science, fluid and combustion physics, global environmental monitoring and many other disciplines. Even with the improved exercise countermeasures and added knowledge of

today, the overhead of spending up to two hours each and every day in space for the sole purpose of exercise is problematic.
ISS crewmembers actively work with strength and conditioning coaches throughout their preflight training. Using exercise monitoring hardware aboard ISS, these same coaches perform inflight assessments of the crew's conditioning while they are in space, and make exercise prescription modifications from Mission Control Houston, as required. Additionally, they oversee the crew's postflight physical rehabilitation, a process which may take several months to restore bone density to critical areas such as the hip and lumbar vertebral bodies.
Armed with an understanding of the whole body, cellular and subcellular processes involved in bone density maintenance in altered gravitational fields, more effective and efficient means to preserve musculoskeletal health is necessary to send humans beyond short stays aboard the ISS: Lunar outposts and expeditions to Mars are even more committing endeavors, and warrant substantial attention.

5. Future directions for research

Despite a reasonable foundation of information, much work is needed to further delineate the impact of microgravity on bones at the cellular and systemic levels. Clearly the best strategy is to conduct experimental in-vivo human studies in space, but limited access to spaceflights and limited time during flight available to dedicate to these studies renders extensive (but necessary) study unachievable[1]. Accordingly, microgravity simulation has been the primary source of basic biological scientific information including most of what has been discussed thus far in this chapter.
On the celular level, simulation can be carried out within the rotating-wall vessel (RWV); a NASA-designed tissue culture bioreactor which simulates microgravity[1]. The bioreactor rotates horizontally such that, at an ideal speed, the contents achieve relative suspension simulating microgravity via dynamic equalibrium of forces---the contained cells / tissues remain in a state of long-term, suspended free-fall. The cells / tissues retain viability by being contained along with cell-specific growth media and oxygenation via active or passive diffusion provided by a silicon rubber membrane. To date however, relatively few studies have been carried out and there is significant need for further study on the cellular level as this level may be the key to differences relative to earthly osteoporosis. Additionally, comparison with studies performed in space will be required to validate the model and to ensure that changes noted are not unique to the system itself---in-vitro cellular behavior does not always mirror real life.
On the system / organism level, research has focused on animal models; most commonly hind-limb unloading and head-down bed rest (which has also been used in human volunteer subjects)[1]. While such models provide some insight into rapid bone loss, they are not fully satisfactory given that they fail to incite the noted cellular changes associated with microgravity and gravitational forces still compress bodily tissues whereas, in true microgravity, there is negative pressure experienced by tissues. It is clear that better models need to be developed.

6. Potential future options for treatment and prevention

Current options for the prevention and treatment of osteoporosis have proven far more successful on earth than in microgravity and this is likely commensurate with the above

noted cellular anomalies encountered. Aggressive exercise by astronauts---recommended at two hours per day of heavy resistance work---has made an impact; however, freeing up time for such activities is difficult given the operational needs during missions and, as space flight expands generally, the baseline cardiovascular capabilities of travelers will be more limited. Additionally, the aforementioned cellular changes render supplements (Calcium, vitamin D, etc.), medications (bisphosphonates, etc.) and hormones (testosterone, etc.) significantly less effective in astronauts despite having a minor effect in microgravity animal models.

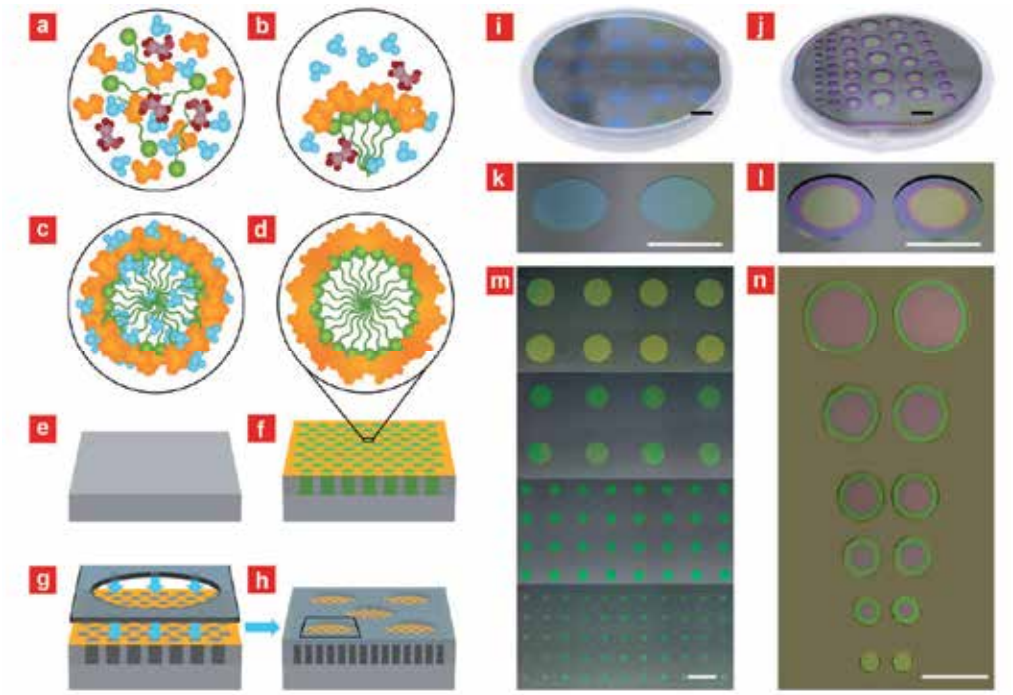
6.1 Diagnostic platforms

The identification of new diagnostic or prognostic biomarkers has been gaining attention in the field of bone disease research leading to significant benefits in terms of efficient and timely treatment. Clearly novel strategies will need to be developed, and directed both at the molecular / cellular and bony systemic levels, and will need to be long lasting and simple to administer. In our minds, the ideal platform for the development of such novel strategies will rest upon nanotechnology. The size of nanomaterials mirrors that of most biological molecules and structures allowing size-matched communication and intervention important in diagnostics and therapeutics at the sub-cellular level and felt to be the source of bone cell dysfunction in microgravity.

In this context, particular emphasis is placed on study of circulating proteome. The proteome represent the functional picture of the state of the cells because it constantly changes through its biochemical interactions with the genome and the environment. Protein turnovers and tissue microenvironment create a rich and heterogenic circulating mixture of protein fragments (low molecular weight peptidome, LMWP) that reflects both physiological and pathological processes. Despite its potential in clinical applications, profiling of the LMWP has proven to be a significant technical challenge because of the extremely high dynamic range of protein concentrations in blood and body fluids. Development of technologies that enable controlled fabrication of structure with nanoscale dimension can address the issues of the intrinsic complexity of the circulating low molecular weight peptidome [12,13]. Our group has developed diagnostic nanochannel-based lab-on-a-chip technologies [Fig 1] that can allow for the detection of the earliest signs of disease, including osteoporosis, using penny-sized discs (satisfying the need for space preservation during space flight). This device is a size-exclusion method based on mesoporous silica thin film chips able to rapidly fractionate, and selectively enrich and protect peptides and proteins from enzymatic degradation. The mesoporous silica chip were produced by the evaporation-induced self –assembly procedure under acidic conditions using triblock copolymers as structural templates [14,15].

Physical properties of mesoporous silica such as pore dimension, pore texture, and chemical surface properties such as charge and further functionalization with selective ligands can be easily controlled and tuned to enhance the ability to detect traces of molecules. The ability to fabricate nanoscale devices and materials with a high degree of precision and accuracy, in combination with the recent advances in mass spectrometry, resulted in a powerful proteomic nanoscale platform for early disease diagnosis [16]. These lab-on-a-chip based diagnostic technologies can be either used as external devices or be implanted in the body of the astronaut. Implantable chips can feature molecularly driven sensors able to measure vital signs and readily respond to specific variation by releasing counteracting molecules. Diagnostics based on readily accessible body fluids can be also used to monitor in real time

the efficacy of therapeutic interventions. In their most complex configuration these implantable devices can be considered as artificial glands that sense the status of the body and adjust to it trying to bring back homeostasis. Nanotechnology based diagnostics offer higher detection capabilities due to the reduction of the size of the sensors, the increase of their sensitivity, the absence of non-specific reactions, and the multiplexing of the multi-scale detectors that allow a wide range of intensities of the signal to be measured.



a-h, Schematic evolution of the chemical composition of the coating solution during the production of a mesoporous silica film. a, Fresh coating solution; b, Formation of micelles; c, Evaporation induced self assembly during spin-coating process; d, Zoomed in view of a pore after aging at elevated temperature. e, Bulk silicon wafer surface; f, Mesoporous silica film on a bulk silicon wafer. e-f, Cross-section of GX6 chip by SEM and TEM imaging respectively (scale bar is 500nm). i-n, images of the different chip surfaces and of the different masks that define the spotting areas.

Fig. 1. Production and assembly of MSC for proteomic applications

6.2 Delivery systems

We developed novel silicon-based theranostic nanoparticles [17-19] that have been used to achieve long-term, controlled, and targeted release of proteins and drugs that help halting or reversing osteoporosis. Among the molecules tested bone morphogenetic proteins (BMPs---which are differentiation factors that facilitate the transition of mesenchymal stem cells to osteoblasts thereby encouraging bone formation) and bisphosphonates (which inhibit osteoclasts mediated bone resorption). The finely-tuned, extended, local delivery allowed by the use of these particles means that a single treatment can be administered pre-flight with effects felt for months on end (no need to 're-dose') and might prove to prevent or treat osteoporosis of microgravity. Nanoporous silica and PLGA composites are capable of

releasing molecules in a burst or steady fashion over the course of days, weeks, or even months. These systems can also be tuned to release their payload in response to environmental stimuli (pH, temperature, blood concentrations, exposure to radiation, bone degeneration, etc.). The local delivery of antibiotics, dexamethasone, and growth factors (BMP-2) to the bone defect areas by PLGA/pSi microspheres reduced inflammation and stimulated new bone formation whilke simultaneously fighting bacterial growth. A wide variety of therapeutic and imaging agents have been successfully loaded into and released from pSi particles such as steroids [21], hormones [22], proteins[23], cancer drugs [24], or even secondary drug delivery vehicles including iron oxide nanoparticles [25], quantum dots, liposomes [26] and carbon nanotubes loaded with therapeutic drugs[18,27] to the diseased areas. In order to achieve the level of control on the release dynamics, it is possible to tailor both the pore size of the pSi during particles' fabrication or vary polymer type, molecular weight and density. Finally, the overall size of the polymer/pSi composites can also be tuned from nano level to micro level to suit certain applications by changing the polymer concentration, surfactant concentration, or the stirring speed. This hybrid system not only can reduce or abolish burst release, and prolong release kinetics, but also protect biomolecules from denaturation both during the drug loading process and while implanted in vivo. These particles have been successfully tested in different orthopedic tissue engineering applications in small and large animal models of bone fracture repair (manuscripts in preparation).

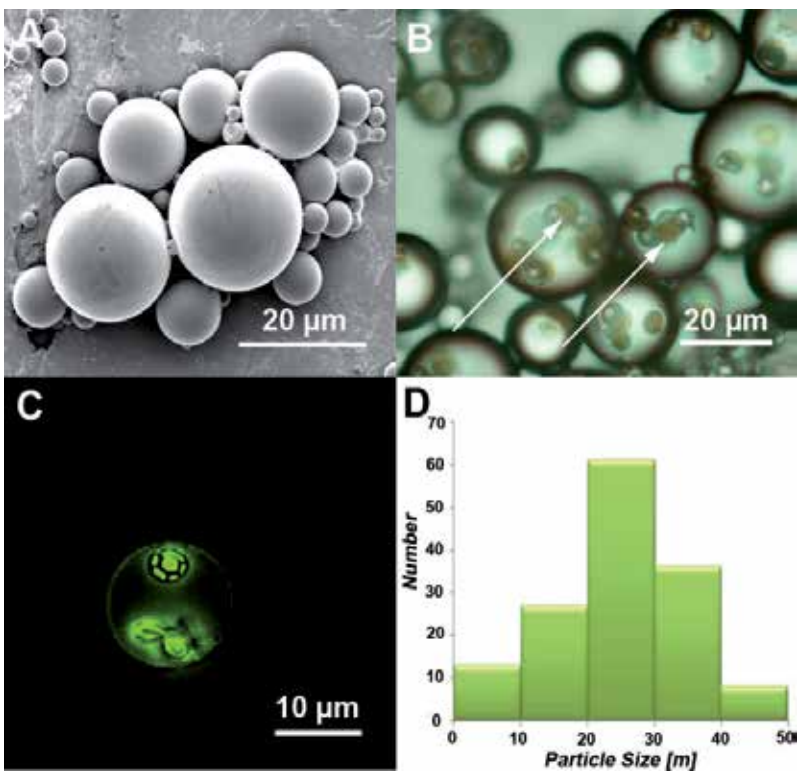


Fig. 2. Scanning electron microscopy (SEM) images of pSi particles reveals (A) uniform shape and size of particles, (B) the pore structure on the surface of the particles, and the (C) front and (D) rear surfaces of the particles.

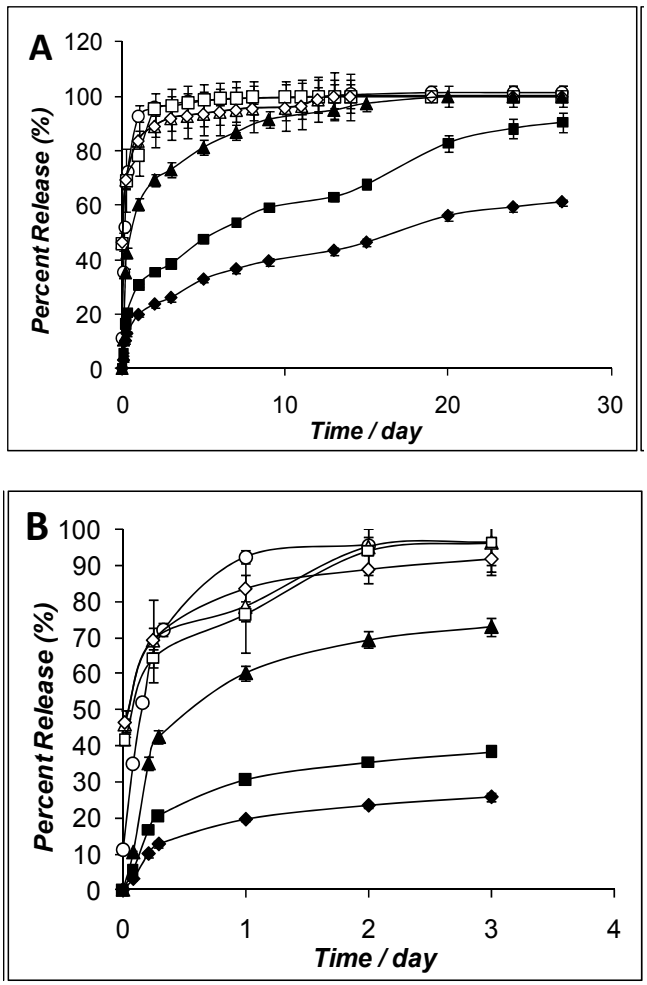


Fig. 3. Release profiles of FITC-BSA from various examined PLGA/pSi microsphere formulations. (A) Total FITC-BSA released over 27 days, (B) first three day release.

6.3 Injectable materials

Beyond these diagnostic and drug-delivery applications, our group has also developed injectable gels that employ nanotechnologies to deliver mesenchymal stem cells, platelet rich plasma, and osteogeneic factors directly to areas of bony weakness. Thus, astronauts identified to have focal osteoporosis of, say, the proximal femur, might be treated by simple focal injection affording the in-vivo, in-situ rapid regeneration of lost bony mass.

These composites have proved their osteogenic capacity in vitro and through in vivo subcutaneous implants where ectopic bone was formed. The use of bio-porogens synthesized from natural and biodegradable materials, encourages bone formation and vascularization in vivo. These porogens particles house and release MSC, recruit endogenous cells and create extracellular matrix, synergistically promoting bone formation

[28]. They also exhibit tremendous viability of MSC after cryo-preservation, allowing for long-term storage of prepared bio-porogens for immediate "on-demand" use in the clinic. Previously, we found that MSC isolated from compact bone (CB) tissue were more frequent in the total cell population and of greater colony-forming and tri-lineage differentiation potential than MSC in bone marrow (BM) (Figure 4).

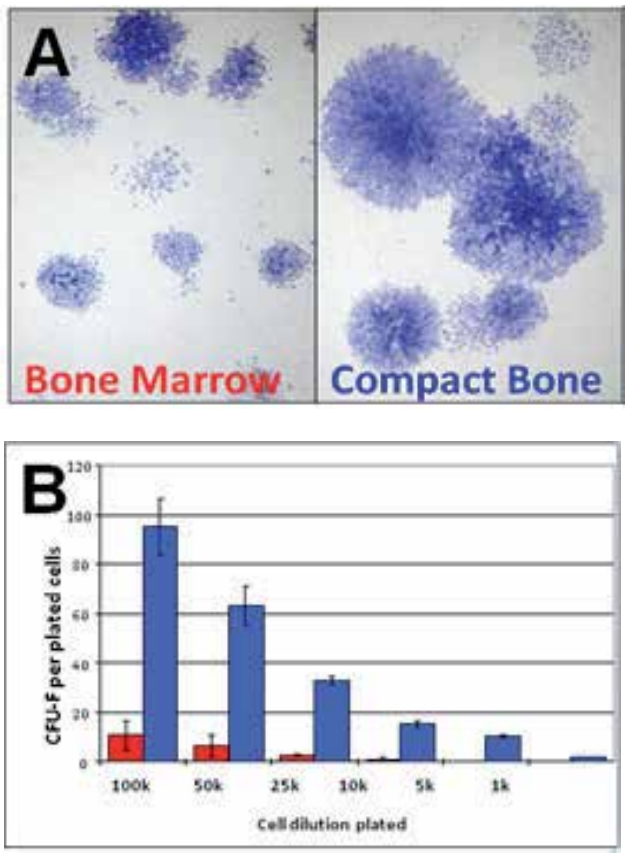


Fig. 4. MSC from compact bone (CB) produce larger and more defined colonies than those from bone marrow (BM) (A). The incidence of MSC from bulk cell populations is also nearly 10x higher in CB than BM (B).

All these biomaterials were based on the unique combination of I) nanostructured biomaterials able to mimic the extracellular matrices of either bone or cartilage with II) chemical and biochemical cues able to direct, control and preserve the phenotypes of both osteoblasts in their histological compartments. These biomaterials are made available as injectable hydrogel formulations thus reducing surgical invasiveness and improving the accuracy of the delivery to the targeted anatomical sites. Injectable composite hydrogels/pastes can be used for the spinal regions weakened by OP, with appropriate biomimetic and biomechanical characteristics.

These biomaterials can be functionalized and/or doped with chemical (e.g. strontium ions, oxygen transporters/scavangers) and biochemical (e.g. bioactive/biodocking peptides,

genes) agents able to control cell phenotype and activity. Hydrogel formulations to be examined include collagen, gelatin, alginate, self-assembling peptides, or combinations thereof. The nano-features include peptides that bind to integral growth factors such as BMP-2 and VEGF and PRP. The scaffolds may be co-implanted with mesenchymal stem cells obtained from bone marrow and adipose aspirates. Finally, we have developed ways to reinforce biocompatible polymers with nanoparticles / nanowires that greatly increase their strength allowing for the replacement of bulky, heavy metallic devices currently used for fracture repair---the light-weight, injectable polymers ideal for transport on space flights and use for the repair of osteoporotic fractures once they occur.

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15

Neurological Osteoporosis in Disabilities

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1. Introduction

Osteoporosis is characterized by low bone mass and destruction of the micro architecture of bone tissue, resulting in increased bone fragility and susceptibility to fractures (NIH 2001). The clinical usefulness of T-score at disabled people on the recognition of people with low BMD remains unclear according to ranking system of the World Health Organization (WHO 1994). Despite the increased number of risk factors in people with disabilities no guidelines are available on BMD measurements; so it would be more appropriate to use the term low bone mass instead of osteoporosis or osteopenia and also take into account the Z-score obtained from the measurement of bone densitometry which is the number of standard deviations above or below that normally expected for someone of similar age, sex, weight and race in question (Dionyssiotis, 2011c, 2011d).

In disabled subjects there are differences according to the type of injury (i.e. lesion with a level of injury vs. upper motor neuron pyramidal lesion), the type of lesion; complete (an absence of sensory or motor function below the neurological level, including the lowest sacral segment) vs. incomplete lesion (partial preservation of motor and/or sensory function below the neurological level, including the lowest sacral segment), the progression or not of the disease (i.e. progressive multiple sclerosis vs. complete paraplegia), life expectancy, the residual mobility and functionality, the ability to walk and stand (i.e. incomplete paraplegia vs. quadriplegia vs. high-low paraplegia), drug treatment (i.e. frequent corticosteroid therapy in multiple sclerosis vs. long-term therapy with anticoagulants in paraplegia), the degree of spasticity (i.e. flaccid vs. spastic paralysis) and it is necessary to take into account the issue of fatigue and muscle weakness. Depression in these subjects is usual; complicates the proposed treatments and limits mobility. Complete and incomplete disabled differ also in physical abilities. Moreover, subjects with complete injuries have greater bone loss than those with an incomplete injury (Garland et al., 1994) and as has already been shown in Brown-Sequard subjects (incomplete spinal cord lesion) where BMD of the more paretic knee was lower than that of the stronger knee (Lazo et al., 2001).

However, there are also similarities; for example the clinical equivalence of diseases with different physiopathology, location, evolution, etc. A severe form of multiple sclerosis (MS) can result in a wheelchair bound patient having a clinical figure equivalent to spinal cord injury paraplegia. One patient with MS may have better walking gait pattern in comparison with a patient with incomplete paraplegia but may also be unable to walk, bedridden and vice versa (Dionyssiotis, 2011c, 2011d).

In addition the role of factors which do not change, i.e.: race or gender is inadequately clarified. Studies in disabled women debate that bones are more affected compared to disabled men. In chronic spinal cord injured women a tendency to have lower bone mass than men (Coupaud et al., 2009) and higher rates of lower bone mass with lower T-scores compared to women with other disabilities have been reported (Smeltzer et al., 2005).

2. Spinal cord injury

Bone loss in spinal cord injury (SCI) is a multifactorial disease in acute and chronic phase and can be enhanced by the lack of weight bearing, muscular tension on bone or other neural factors associated with the injury. Moreover, differentiation of the sympathetic nervous system after SCI is leading to venous and capillary vascular stasis. Some additional non-mechanical factors to stimulate bone loss include poor nutritional adequacy, gonadal changes and other endocrine disorders (Chantraine 1978; Chantraine et al., 1979b; Jiang et al., 2007; Maimoun et al., 2006).

2.1 Bone mineral density

In individuals with SCI bone loss begins immediately after injury (Bauman et al., 1997; Uebelhart et al., 1995). SCI related bone impairment below the level of injury is much greater compared with other conditions (i.e. age, immobilization, bed rest, lack of gravity environment). A reduction of bone mineral content (BMC) during the first years after the injury of 4% per month in regions rich in cancellous bone, and 2% per month on sites containing mainly cortical bone is reported (Wilmet et al., 1995). According to another study 25 out of 41 patients with SCI (61%) met WHO's criteria for osteoporosis, eight (19.5%) were osteopenic and only eight (19.5%) showed normal values (Lazo et al., 2001). In SCI children (boys and girls) values for bone mineral density (BMD) at the hip were approximately 60% of normal, or had a Z-score that indicated a 1.6-1.8 SD reduction in BMD compared with age- and sex-matched peers (Lauer et al., 2007).

In studies with peripheral quantitative computed tomography (p QCT) in spinal cord injured subjects bone loss in the epiphyses was 50% in the femur and 60% in the tibia, while in the diaphyses of these bones was 35% and 25%, respectively, meaning that bone loss in the epiphyses almost doubled the loss in the diaphyses (Eser et al., 2004). This study also showed that bone loss between trabecular and cortical bone compartment differs in mechanism, i.e. in the epiphyses bone is lost due to the decrease in trabecular, while in diaphysis, the cortical bone density is maintained and bone is lost due to endocortical resorption. In line with the previous study another p QCT study, performed in complete paraplegics with high (thoracic 4-7) and low (thoracic 8-12) neurological level of injury at the tibia, found a loss of trabecular (57.5% vs. 51%, in high vs. low paraplegics, respectively) and cortical bone (3.6% and 6.5%, respectively), suggesting that trabecular bone is more affected during the years of paralysis in comparison with cortical bone (Dionyssiotis et al., 2007). In the same study both paraplegic groups had a similar loss of total BMD (46.90% vs. 45.15%, in high vs. low paraplegics, respectively) suggesting that a homogenously deficit pattern occurs in the epiphyseal area, especially in the group of low paraplegics because the central and the peripheral of the cross sectional area of bone were similarly affected. On the contrary, in high paraplegics' group trabecular bone loss was higher suggesting an increasing endocortical remodeling keeping the total BMD similar. Concerning cortical geometric properties the results had shown an increased endosteal circumference between

both paraplegic groups vs. controls leading to reduction of cortical thickness, 19.78% vs. 16.98% in paraplegic groups respectively, whereas periosteal circumference was comparable to controls (Fig. 1).

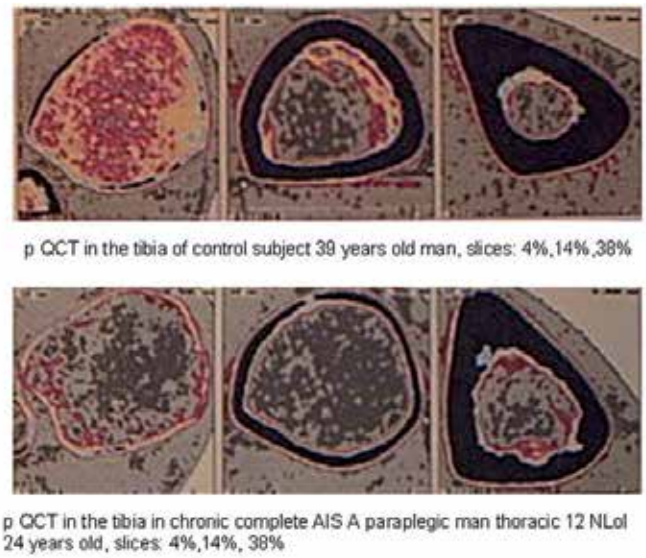

Fig. 1. Peripheral quantitative computed tomography (p QCT) tibia slices in control (a) and paraplegic subject (b), (scanner XCT 3000 Stratec, Medizintechnik, Pforzheim, Germany). Areas in red represent trabecular bone, while areas in grey represent fat; pQCT allows the measurements of true volumetric densities at a minimum exposure to X-rays, assess cortical and trabecular bone density separately as well as to evaluate the geometrical properties of long bones non-invasively, adapted from Dionyssiotis, 2011c, 2011d, with permission.

Regarding tetraplegic patients statistically significant differences were found in BMD of the spine, trochanteric region and upper limbs between paraplegic and tetraplegic patients but not in the femoral neck, pelvis, and lower extremities (Tzuzuku et al., 1999). Indeed, the effects on spinal BMD differed from previously published work in which the investigation was mainly focused in paraplegics (Biering-Sorensen et al., 1988, 1991; Leslie & Nance, 1993).
The importance of mechanical loading and site specificity to maintain or increase BMD is already shown (Lanyon, 1986). According to bone loss there are some interesting features in spinal cord injured subjects; demineralization is area dependent, occurs exclusively in the areas below the level of injury (Dauty et al., 2000), affecting mainly paralyzed extremities and increasing from proximal to distal regions i.e. in paraplegics weight bearing skeleton regions, as the distal end of femur and proximal tibia, which are rich in cancellous bone, while region of the diaphysis of the femur and tibia, rich in cortical bone is reserved (Eser et al., 2004; Kiratli et al., 2000; Dionyssiotis et al., 2007). Moreover, bone loss between trabecular and cortical bone compartment differs in mechanism, i.e. in the epiphyses is due to decrease in trabecular but in diaphysis cortical bone is maintained and bone is lost through endocortical resorption by reducing cortical wall thickness (Dionyssiotis et al., 2007; Eser et al., 2004).

Women with disabilities have a higher risk of losing bone mass compared to men because of the inevitable reduction in estrogen levels that occurs at menopause. Findings that women with serious disabilities have low bone density are not surprising and are probably related to the lack of activity (reduced mobility, reduced loading on bone) and worsening of the disability. Regarding women with complete SCI, the initial bone loss in the lumbar spine is negligible. Post injury over a period of years BMD in SCI women is maintained or increases compared with non-injured age-matched women, in whom BMD decreases during aging (Dionyssiotis, 2011c).

2.2 Duration of paralysis and bone steady state

The duration of paralysis affects the degree of bone loss in regions below the level of injury. A study of 21 men with SCI with an average duration of 10.6 years, using dual-energy X-ray absorptiometry (DXA), expressed at various levels of injury an inverse relationship between BMD in the legs and the duration of the lesion (Clasey et al., 2004), while others found a weaker relationship regarding the microarchitecture of the distal end of tibia (Modlesky et al., 2004).

In a study which included paraplegics with duration of paralysis of 14 ± 11.5 years a positive correlation between the duration of paralysis and the degree of bone loss was found (Eser et al., 2004). The length of immobilization in the acute posttraumatic period increased bone loss in the legs, particularly in the proximal tibia; over 50% of bone mass was lost (in the affected areas) in the period of ten years after the injury (Dauty et al., 2000). When subjects categorized depending on the length of the lesion (0-1, 1-5, 6-9, 10-19, 20-29, 30-39, 40-49, and 50-59 years after the injury), in all age groups bone loss to the hip area occurs a year after the injury (Szollar et al., 1998).

Using DXA and QUS (quantitative ultrasound) measurements in 100 men with SCI, aged 18 to 60 years, it was found that bone density decreases over time in all measured points, while bone loss followed a linear pattern in the femoral neck and distal epiphysis, stabilized within three years after the injury. On the contrary, Z-scores of the distal region of the diaphysis of the tibia continued to decrease even beyond ten years after the injury (Zehnder et al, 2004). Duration of paralysis related bone loss in the legs of monozygotic twins with chronic paraplegia in comparison with their able-bodied co-twins has been also reported (Bauman et al., 1999).

The results of a comparison of chronic complete paraplegic men vs. controls in another study found a reduction of BMD in paraplegics' legs independent of the neurological level of lesion. BMD of the legs was negatively correlated with the duration of paralysis in the total paraplegic group, but after investigation according to the neurological level this correlation was due to the strong correlation of high paraplegics' legs BMD with the duration of paralysis, suggesting a possible influence of the neurological level of injury on the extent of bone loss (Dionyssiotis et al., 2008). A significant inverse relationship between percentage-matched in BMD leg, arm and trunk values and time since injury was found when varying levels of SCI were analyzed (Clasey et al., 2004).

Studies are supporting the concept of a new bone steady state at 16-24 months after injury, especially for bone metabolic process (Bauman WA 1997; Demirel et al., 1998; Szollar et al., 1998), but BMD decreases over the years at different areas and is inversely related to the time of the injury, which means continuous bone loss beyond the first two years after the injury (Coupaud et al., 2009; Dionyssiotis et al., 2008; Eser et al., 2004) (Fig. 2).

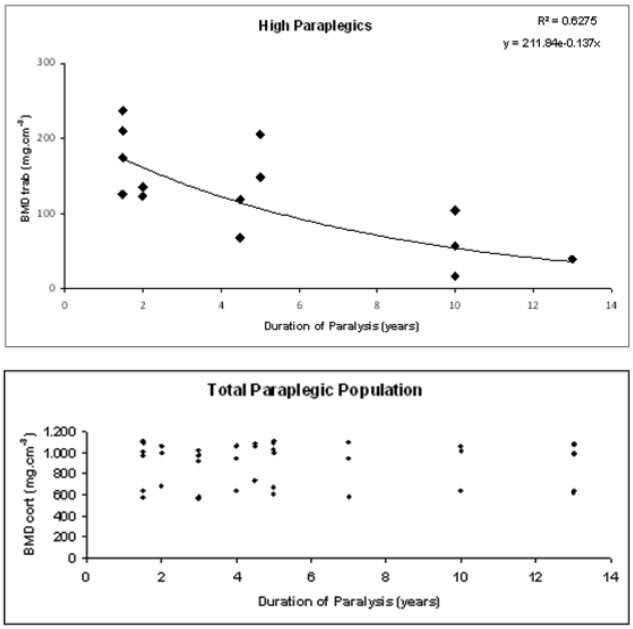

Fig. 2. The duration of paralysis was inversely related with trabecular bone loss in spinal cord injured subjects. Exponential correlation between volumetric trabecular bone mineral density BMD trab and duration of paralysis in high paraplegics was found to fit best. On the contrary no significant decrease in BMD cort of the diaphyses was found in total paraplegic group. BMD parameters were measured by pQCT in 31 paraplegic men in chronic stage (>1.5 years of injury). Spinal cord injury paraplegic men were allocated into 2 subgroups based on the neurological level of injury; subgroup A (n=16, Thoracic (T)4-T7 neurological level of injury) and subgroup B (n=15, T8-T12 neurological level of injury). BMDtrab: BMD trabecular; BMDcort: BMD cortical; (adapted from Dionyssiotis et al., 2011a, with permission).

The role played by factors such as race or gender of patients is not yet clear documented, but studies indicated more loss in women than men (Garland et al., 2001). Loss of bone is closing fracture threshold from 1 to 5 years after injury (Szollar et al., 1998) and risk factors for fractures after spinal cord injury are gender (women are more at risk than men), age and duration of injury (increasing age and duration of injury increases the risk of fracture with a statistically significant increase in 10 years after injury), the type of injury (complete SCI subjects have more fractures than incomplete), low body mass index (BMI) and low bone density in the tibia (Garland et al., 2004a,b; Garland et al., 1992; Lazo et al., 2001).

2.3. The role of central nervous system

2.3.1 Sympathetic denervation in SCI

Spinal cord injury is a dynamic process that is related to alterations in both the central and peripheral sympathetic nervous system (SNS). Sympathetic denervation in SCI may cause arteriovenous shunts and a slowdown of intraosseous blood flow, thus increasing bone resorption (Chantraine et al., 1979). With high-level spinal cord lesions the SNS is disproportionately involved when compared with the parasympathetic nervous system. In a complete high-level SCI, functioning in the isolated spinal cord below the lesion becomes

independent of supraspinal control and has been termed ″decentralization″ of the SNS (Karlsson et al., 1998).
Loss of supraspinal control leads to dysregulation of those homeostatic mechanisms normally influenced by the SNS through loss of facilitation or lack of inhibition (Teasell et al., 2000). Today there is clinical evidence that the sympathetic regulation of bone does exist in humans and plays a clinically important role in diseases characterized by excessive sympathetic activity (Schwartzman, 2000). The scientific finding about sympathetic innervations of bone tissue (Takeda et al., 2002; Kondo et al., 2005) and its role in the regulation of bone remodelling is of major interest in situations where uncoupling between osteoclasts and osteoblasts occurs (Levasseur et al., 2003).

2.3.2 Spasticity

Controversial results have also been reported regarding the effect of spasticity on BMD in SCI paraplegics. A cross-sectional study of 41 SCI paraplegics reported less reduction of BMD in the spastic paraplegics SCI patients compared to the flaccid paraplegic SCI patients (Demirel et al., 1998). Others reported that spasticity may be protective against bone loss in SCI patients, however, without any preserving effect in the tibia (Dionyssiotis et al., 2011; Eser et al., 2005). A possible explanation for that could lie in the fact that in the present study all paraplegics were above thoracic (T)12 level with various degrees of spasticity according to the Ashworth scale. In addition, muscle spasms affecting the lower leg would mainly be extension spasms resulting in plantar flexion thus creating little resistance to the contracting muscles. Furthermore, the measuring sites of the tibia did not include any muscle insertions of either the knee or the ankle extensor muscles (Dionyssiotis et al., 2011a; Dionyssiotis, 2011c). Other investigators also have not been able to establish a correlation between BMD and muscle spasticity (Lofvenmark et al., 2009).

3. Multiple sclerosis

Reduced mobility has been implicated as an important factor in bone loss in patients suffering from multiple sclerosis (MS) and it seems to greatly influence the BMD of the femur. However, the high proportion of ambulatory patients with bone loss suggest additional non-mechanical factors (Cosman et al., 1998; Dionyssiotis, 2011b).
There is a high incidence of vitamin D deficiency in MS patients and is determined by levels of 25-hydroxy vitamin D <20ng/ml (Nieves et al., 1994). The reasons might be due to a combination of low dietary vitamin D intake and avoiding of sun exposure, and that because of MS symptoms may worsen after sun exposure (fatigue-related heat) leading these patients to avoid sun. Low testosterone alone in these populations does not explain bone loss and no clear effect of smoking or alcohol abuse to decreased bone mass could be established (Weinstock-Guttman et al., 2004).
Glucocorticoid (GC)-induced osteoporosis (OP-GC) is the main type of secondary osteoporosis (Canalis et al., 2004; Canalis et al., 2007; Lakatos et al., 2000; Mazziotti et al., 2006; Schwid et al., 1996; Shuhaibar et al., 2009). The mechanism is that excess GC causes a rapid and significant damage to bone quality. Now days we know that GCs act direct on bone mainly to the stromalosteoblastic lineage and at high concentrations alter differentiation, survival, and function of them causing a shift from osteoblastic to adipocytic differentiation of precursors; inducing apoptosis of mature osteoblasts; and inhibition of

synthesis and secretion of bone components (Manolagas, 2000; Pereira et al., 2002). Finally, GCs promote ostoclasts and stimulate bone resorption (Weinstein et al., 2002). The mechanisms of GCs action in bone has been studied extensively. In patients receiving chronic per os GC, bone loss is admitted rapidly and is evident within 6 or even 3 months (Cosman et al., 1998). A study investigated the effect of intravenously (i.v.) administration of glucocorticoids in MS patients found no clear effect on bone loss: on the contrary they reported an increase in BMD of the lumbar spine (Schwid et al., 1996). Prolonged treatment with glucocorticoids results in increased risk of fractures, evident at 3 months, regardless of changes in BMD. High dose, short-term i.v. treatment with GCs leads directly to reduction of bone formation and increased bone resorption, as indicated by markers of bone turnover (De Vries et al. 2007; Van Staa et al., 2000). Osteopenia not osteoporosis was significantly more frequent in patients with MS compared with controls, especially in women who received high dose methylprednisolone pulses (HDMP) in relapses period making important the regularly monitoring of BMD in these patients. The authors concluded that disability and the subsequent immobilization osteoporosis is the more serious factor in this group and treatment with repeated HDMP pulses did not cause osteoporosis in MS subjects followed-up for almost 8 years unlike chronic corticosteroid therapy which induces osteoporosis and/or recovery of BMD is permitted without permanent skeletal damage (Zorzon et al., 2005). The lack of physical activity exacerbates osteoporosis. All MS patients should be considered high risk for osteoporosis. Prevention with calcium rich foods and dietary supplements containing vitamin D and antiosteoporotic drugs is necessary for these patients. Particular attention should be paid to transfers and falls prevention in this population to prevent fractures which occur easily and heal slowly (Cattaneo et al., 2007; Dionyssiotis, 2011b).

In osteoporosis molecular mechanisms leading to bone loss are inadequately explained. There is evidence of interaction between bone and immune system. T cells' activity could stimulate bone loss under certain circumstances such as estrogen deficiency. Women with post-menopausal osteoporosis have higher T cell activity than healthy post-menopausal subjects which could be also the case in inflammatory or autoimmune disorders like MS: receptor activator of nuclear factor kappa B ligand (RANKL) stimulates osteoclastogenesis and the same do cytokines, such as TNF-*a*, IL-1, or IL-11, all produced by T-cells activation, leading to bone destruction. On the contrary osteoprotegerin (OPG) is an osteoclastogenesis inhibitory factor preventing the function from RANKL. A balanced system of RANKL/OPG regulates bone metabolism. In MS this system is disturbed in favour of RANKL (Zhao et al., 2008; Kurban et al., 2009).

4. Stroke

Disuse has been suggested as the main cause for loss of bone mass in patients immobilized because of stroke (Takamoto et al., 1995). However, this was not confirmed in a prospective study, in which only weak associations between bone loss and motor function, activities of daily living (ADL), or ambulation were found (Ramnemark et al., 1999a). This could be explained by the selected severely affected patients, but it does raise questions about other risk factors for the development of hemiosteoporosis apart from paresis and immobilization (Ramnemark et al., 1999b).

The critical role in pathogenesis of osteoporosis is attributed to hormonal processes and osteoporosis itself is often defined as generalized skeletal disorder. Findings of tibial bone

changes in hemiplegic patients are not compatible with this view. The adaptations are found in trabecular bone in the epiphysis as well as in cortical bone in the diaphysis. They represent an individually different distribution of local changes which can be explained by the feedback principles of the muscle-bone-unit, in which bone strength is controlled by the muscle forces that act upon the bone. Muscle forces acting habitually on the paretic limb are considerably less than on the opposite side. This reduction of forces reduces the strain on bones. This leads to loss of bone mass and bone strength (Runge et al., 2004).
Determinants of bone mineral loss have been identified as duration of hemiplegia-induced immobilization and severity of palsy (Sato, 1996). A rapid and pronounced loss of BMD in the paretic extremities that progressed during the first year after stroke (Ramnemark et al., 1999a) more pronounced during the first few months after stroke onset (Hamdy et al., 1993). The lower extremities lost BMD bilaterally, but the losses were significant after 12 months in the affected femur, proximal femur and trochanter. In immobile patients, this could explain the loss of BMD in the nonaffected leg as compared with the nonaffected arm, which even increased in BMD, probably due to increased compensatory activity (Ramnemark et al., 1999a).
Hemiosteoporosis has previously been described as being caused by disuse and vitamin D deficiency (Sato et al, 1996), and in a randomized study a significant decrease in the rate of bone loss in stroke patients with a mean duration of 4.8 years after stroke when supplemental vitamin D was given (Sato et al., 1997). Bone mineral loss was more pronounced in the upper than in lower limbs, and the difference between sides was more marked in long-standing poststroke hemiparesis. The upper versus lower difference may reflect that hemiparesis from stroke is commonly more severe in the upper limb. Notably, BMD on the nonhemiplegic side is intermediate between that for the hemiplegic side and that in control subjects. The decrease in mobility of the intact limb, resulting from stroke-related need for assistance with activities of daily living, presumably results in mild osteoporosis paralleling the patient's overall degree of immobilization (Sato et al., 1998, 2000).

5. Myelomeningocele and cerebral palsy

Previous studies suggest that the level of neurological injury and mobility affect BMD in myelomeningocele (MMC). Studies concluded that loading of the lower limbs rather than child's potential ability to walk because of the level of neurological lesion or residual motor capacity of lower limbs is a prognostic criterion for the BMD (Apkon et al., 2009; Ausili et al., 2008; Quan et al., 1998). This theory is probably challenged by other studies that revealed low values of forearm BMD in individuals and indicate that in this patient osteoporosis can be caused by neurogenic and metabolic mechanisms. The fact is that these patients are loading the arms through the use of crutches and wheelchairs and BMD values in the upper extremities are expected to be higher in relation to immobilized people (Quan et al., 1998). Subjects with MMC may have hypercalciuria associated with immobilization and an additional risk factor for osteoporosis in these patients group (Quan et al., 2003). Others support that low-energetic fractures in MMC children may result from metabolic disturbances that are a consequence of excessive renal calcium loss or excessive fatty tissue content (Okurowska-Zawada et al., 2009).

Children with cerebral palsy (CP) are growing slowly. The impact of this altered growth on skeletal development and bone density is a difference in linear growth which becomes more accentuated over time compared with their typically growing peers. In addition, as growth slows, the bone mineral density also falls further outside the normal range (Houlihan et al., 2009). Significantly decreased bone density is virtually universal in non-ambulatory children with moderate to severe CP after the age of 10 years (Henderson et al., 2002); Bone-mineral content and density were measured in a study by dual energy X-ray absorptiometry in the proximal femur, femoral neck, and total body of nutritionally adequate children (n=17; 11 girls, six boys; aged 7.6 to 13.8 years) with spastic cerebral palsy (CP) and found that non-independent ambulators had lower z scores for total body BMD, femoral neck BMD, and BMC than independent ambulators (Chad et al., 2000). The potential causes of deficient bone mineralization in this population are multiple, including poor nutrition and abnormal vitamin D metabolism. Findings from recent studies (Shaw et al. 1994, Henderson et al. 1995, Wilmhurst et al. 1996) suggest that non-nutritional factors, such as ambulation, may contribute to the alterations in body composition observed in children with CP.

5.1 Interventions to prevent bone loss

5.1.1 Weight bearing activities-cycling-body weight supported treadmill

The effect of standing in bone after SCI has been investigated by many researchers. A beneficial effect on bone mass using passive mechanical loading has been shown on preservation of bone mass in the region of the femoral shaft, but not at the proximal hip of standing and non-standing patients and relatively better-preserved densities in patients standing with braces than in those using a standing frame or standing wheelchair (Goemaere et al., 1994). A slower rate of bone loss in paraplegic subjects who did standing was expressed in a prospective study of 19 patients in acute SCI phase participated in early standing training program showed benefits concerning the reduction of cancellous bone loss compared to immobilized subjects (de Bruin and others 1999; Frey-Rindova and others 2000), while no correlation for passive standing-training to bone status was found in another p QCT study (Eser et al., 2005). Protection afforded by standing in the femoral diaphysis stands in contrast with the loss of bone in the proximal femur. This suggests that the transmission of forces through trabecular and cortical bone varies; so the less effective strain for the initiation of bone remodeling reaches faster cortical bone (Frost, 1992, 2001, 2003). Others also supported the concept of different strain thresholds bone remodeling control (Gutin & Kasper, 1992; LeBlanc et al., 2007; Smith et al., 2009). There is level 2 evidence (from 1 non-randomized prospective controlled trial) that Functional Electrical Stimulation (FES) - cycling did not improve or maintain bone at the tibial midshaft in the acute phase (Eser et al., 2003). Moreover, there is level 4 evidence (from 1 pre-post study) that 6 months of FES cycle ergometry increased regional lower extremity BMD over areas stimulated (Chen et al., 2005). Body weight supported treadmill training (BWSTT) did not alter the expected pattern of change in bone biochemical markers over time and bone density at fracture-prone sites (Giangregorio et al., 2009).

5.1.2 Whole body vibration

At a meeting of the American Society for Bone and Mineral Research results of a small randomised, placebo-controlled study among 20 children with cerebral palsy who used a similar, commercially available vibrating platform for 10 min per day, 5 days per week for 6 months were reported (Ward et al., 2001). A significant increase in tibial, but not lumbar-

spine bone density in the treated group was found despite the simplicity, short duration of the "vibration", the young age of the children and the poor compliance (Eisman, 2001).

Fig. 3. Weight bearing in disabled subjects; using standing frames, functional walking with orthoses between bars and crutches, even push-ups in the wheelchair (in case of multiple sclerosis with a clinical equivalent like tetraplegia) bone can be loaded and bone loss rate would be slower (unpublished photos of Dionyssiotis Y).

After 6 months of whole body vibration (WBV) therapy in twenty children with cerebral palsy (age 6.2 to 12.3 years; 6 girls) randomized to either continue their school physiotherapy program unchanged or to receive 9 minutes of side-alternating WBV (Vibraflex Home Edition II®, Orthometrix Inc) not effect on areal BMD at the lumbar spine was observed, while areal BMD seemed to decrease somewhat in the cortical region of the femoral diaphysis. Authors explained that mechanical stimulation increases intracortical bone remodeling and thereby cortical porosity; moreover changes occurred in ways that are not reflected by areal BMD, but might be detectable by more sophisticated techniques such as such as peripheral quantitative computed tomography (Ruck et al., 2010). Low-intensity vibration (LIV) has shown to be associated with improvement in bone mineral density in post-menopausal women and children with cerebral palsy. Seven non-ambulatory subjects with SCI and ten able-bodied controls underwent transmission of a plantar-based LIV signal (0.27 +/- 0.11 g; 34 Hz) from the feet through the axial skeleton as a function of tilt-table angle (15, 30, and 45 degrees). SCI subjects and controls demonstrated equivalent transmission of LIV, with greater signal transmission observed at steeper angles of tilt which supports the possibility of the utility of LIV as a means to deliver mechanical signals in a form of therapeutic intervention to prevent/reverse skeletal fragility in the SCI population (Asselin et al., 2011).

Fig. 4. The Galileo Delta A TiltTable offers a wide variety of applications from relaxation to muscle training for a diverse range of patients who are unable to stand without support. The motor driven adjustable tilt angle of the Galileo Delta TiltTable (90°) allows vibration training with reduced body weight from 0 to 100%. This is ideal for deconditioned and disabled patients for gradually increasing training weights up to full body weight. System for application in adults (max. body height: 1.90 m) and children (max. body height: 1.50 m).The Galileo Delta A TiltTable is exclusively available from the manufacturer Novotec Medical GmbH., (with permission).

5.1.3 Drugs

Calcitonin in varying doses and methods of administration has given variable results in paraplegia (preferred dosage regimen, treatment duration, and administration route for adequate efficacy in SCI patients' remains unclear) (Chantraine et al., 1979a; Minaire, 1987). Likewise, the outcome using bisphosphonates has been variable. Etidronate produced long-term benefit in lower limb bone mineral density (BMD) in selected walking SCI patients (Roux et al., 1998); whereas tiludronate appeared effective in reducing bone resorption and preserving bone mass in a histomorphometric study in 20 paraplegic patients (Chappard et al., 1995). Intravenous pamidronate has been shown to attenuate bone loss in SCI and normalize serum calcium in immobilization hypercalcemia (Bauman et al., 2005). Alendronate (1000 times more potent than etidronate), in an open observational study, reversed BMD loss in men with established SCI increased both axial and trabecular bone density and has proven efficacy and safety in men treated for osteoporosis, prevents hypercalciuria and bone loss after bed rest and lower leg fracture (Moran de Brito et al., 2005; Zehnder et al., 2004). Six months after using zolendronic acid in the treatment group BMD showed differences in the response to treatment between the mixed trabecular/

cortical regions (narrow neck and intertrochanteric) and the purely cortical shaft. With respect to cross-sectional geometry, bone cross-sectional area and sectional modulus (indices of resistance to axial and bending loads, where higher values would indicate a positive effect of treatment) increased at the hip and buckling ratio (an index of the instability of thin-walled cross sections, where lower values would suggest that the treatment is improving stability) decreased consistent with improved bone outcomes; at 12 months, narrow-neck femur values declined and intertrochanteric and femoral shaft BMD was maintained vs. placebo group which showed a decrease in bone outcomes and an increase in buckling ratio at the hip at 6 and 12 months, while with respect to bone prevention 4 mg i.v. were effective and well-tolerated to prevent BMD loss at the total hip and trochanter for up to 12 months following SCI (Bubbear et al; Shapiro et al., 2007).

Clinical examination and management of bone loss in SCI	
• history of the patient (co morbidities, neurologic complications, use of drugs which impair bone metabolism, alcohol, smoking and information about the level of injury, duration of paralysis, immobilization period, onset of rehabilitation, use of assistive devices and orthoses).	• pharmacological treatment with bisphosphonates p.os and i.v. that have been studied in patients with spinal cord injuries and had positive effects on bone parameters. • Use of calcium supplements (monitoring renal function) and vitamin D.
• anthropometric parameters (age, weight, body mass index, BMI) • clinical examination (level of injury according to American Spinal Injury Association Impairment Scale, AIS) and assessment of spasticity)	• Education on falls prevention • Counseling regarding osteoporosis and related factors and identification of fractures in regions of impaired sensation.
• imaging (bone densitometry by DXA at the hip and spine, and if possible, p QCT at the the tibia or femur)	• physical therapy including: a) range of motion exercises, b) loading of the skeleton to reduce bone loss, d) therapeutic standing-walking with orthoses, e) passive-active cycling
• measurement of bone turnover indices in the serum (parathyroid hormone, alkaline phosphatase, calcium, vitamin D, PINP molecule, osteocalcin) and urinary excretion of 24 hour (calcium, hydroxyproline, aminoterminal (NTx) and carboxylterminal (CTx) intermolecular cross-linking domain of bone type-1 collagen), which provide a good indicator of bone resorption.	• dietary interventions to improve dietary intake of calcium and nutrition indices.

Table 1. An algorithm for the screening and management of osteoporosis in subjects with spinal cord injury (should be read top to bottom starting with the left column); adapted from: Dionyssiotis Y. (2009). Bone loss in paraplegia: A diagnostic and therapeutic protocol. Osteoporos Int Vol. 20 (Suppl 1):S23-S176 (with permission).

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16

Post-Transplantation Bone Disease

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1. Introduction

Solid organ or stem cell transplantation is a well established procedure in the treatment of end-stage diseases (renal disease, chronic liver failure, end-stage pulmonary disease, heart failure). Improved outcome for these patients has allowed us to study some of the complications. One of these is metabolic bone disease, which can hinder their long-term survival and quality of life. In this chapter we have review our current understanding of the pathophysiology of bone loss before and after solid organ transplantation, and review recommendations for the prevention and treatment of osteoporosis in patients accepted into organ transplantation programs. There are a number of risk factors contributing to bone loss in these patients: hypogonadism, vitamin D deficiency, malabsorption, low body weight, physical inactivity, excessive use of tobacco or alcohol and immunosuppressive therapy. Management of pretransplant risk factors has improved, resulting in better bone mineral density (BMD) levels before transplantation (Guichelaar et al., 2006). After transplantation, rapid and marked bone loss is observed in the first 3-6 months. The speed of the bone loss suggests that corticosteroids are heavily involved. Greater bone loss at vertebral and hip sites and high rates of incident fragility fractures have been reported (Leidig-Bruckner et al., 2001).

2. Pathogenetic factors

Many factors contribute to the pathogenesis of osteoporosis after organ transplantation. These include bone disease preceding transplantation, immunosuppressive medications, nutritional and lifestyle factors, and derangements of the parathyroid-calcium-vitamin D and the pituitary gonadal axes (Table 1). However, specific pathophysiological features can also be found in different forms of end-stage diseases.

2.1 Pre-existing bone disease

2.1.1 Kidney disease

End-stage renal disease (ESRD) is associated with a form of bone disease that is generically referred as "renal osteodystrophy". Many mechanisms are involved in its pathophysiology including calcitriol deficiency, hypocalcemia, hyperphosphatemia, secondary hyperparathyroidism, metabolic acidosis, and aluminum overload. Kidney transplantation will improve many aspects of renal osteodystrophy, but parathyroid hyperplasia may not regress even when normal kidney function returns.

General Risk factors
Vitamin D Deficiency and secondary Hyperparathyroidism
Hypogonadism
Inactivity / Immobilization
Poor Nutrition
Low body weight
End Stage Renal Disease
Secondary hyperparathyroidism
Adinamic bone disease
Osteomalacia
Mixed Uremic disease
Metabolic Acidosis
Long term hemodyalysis
Medications: loop diuretics, heparin and warfarin.
Diabetic nephropathy
β-microglobulin amyloidosis
End-Stage Lung disease
Smoking
Chronic use of glucocorticoids
Hypercapnia
Hypoxia
Pancreatic insufficiency (cystic fibrosis).
Failure to attain peak bone mass (in patients who have cystic fibrosis).
Heart failure
Mild renal insufficiency
Medication: loop diuretics, heparin and warfarin.
Failure to attain peak bone mass (in patients with congenital heart disease).
End-Stage Liver Disease
Alcohol abuse
Cholestatic liver disease
Bone marrow transplant recipients
Chronic use of glucocorticoids
Chemotherapy
Growth hormone deficiency (in children)

Table 1. Risk Factors contributing to bone fragility before transplantation

There are several histological subtypes of renal osteodystrophy. The most common is osteitis fibrosa, characterized by increased bone turnover and typically associated with high serum PTH levels (secondary hyperparathyroidism). Osteomalacia is the least common type, with low bone formation and accumulation of unmineralized osteoid. A mixed disease of both, combining increased resorption and increased osteoid, can also exist. Adynamic bone disease is characterized by low bone formation without evidence of fibrosis.
Compared to the general population ESRD patients are 4.4 fold more likely to have a hip fracture and the prevalence of vertebral fracture is 21% higher (Alem et al., 2000). Some of the risk factors for fractures in general population are also seen in patients with renal osteodystrophy: older age, female gender, or low body weight. Specific risk factors in end-stage renal patients are duration of dialysis and peripheral vascular disease (Sethman-Breen et al., 2000).Although BMD in patients with renal osteodystrophy tends to be lower in cortical sites (forearm and hip) than cancellous sites (spine), there is not a clear relationship between the different histological types of renal osteodystrophy and bone density (Gerakis et al., 2000). Furthermore, BMD does not consistently predict fractures after kidney transplantation (Grotz et al., 1994). It is important to note that measurement of bone mineral density (BMD) and WHO criteria cannot be used to diagnose osteoporosis in patients with ESRD. This is because any of the several possible histological forms of renal bone disease may all be associated with low, normal or even elevated BMD.

2.1.2 Lung disease

Patients who are candidates for lung transplantation are highly likely to have osteoporosis before surgery. A retrospective study in patients with diffuse parenchymal lung disease referred for lung transplantation revealed that 30% and 49% of patients had lumbar or femoral osteoporosis respectively (Shane et al., 1996). Other authors found osteoporosis in 50% of the patients at lumbar spine and 61% at femoral neck (Tschopp et al., 2002). Several risk factors such as hypoxemia, malnutrition, vitamin D deficiency, smoking, decreased immobility and low body weight are involved. Cystic fibrosis is associated with additional risk factors such as hypogonadism, inflammatory bone-resorbing cytokines and pancreatic insufficiency that may impair the absorption of calcium and Vitamin D. In addition, most of the patients who undergo lung transplantation have experienced prior glucocorticoid therapy (Tschopp et al., 2002).

2.1.3 Cardiac disease

Osteoporosis and osteopenia are common in patients with severe congestive heart failure (CHF). Lumbar spine osteopenia has been found in 43% of patients, and spine osteoporosis (T-score ≤ -2.5 or Z-score ≤-2.0) in 12-40% of patients. Biochemical markers of bone turnover suggest the presence of increased bone resorption. Involved factors that contribute to bone loss include low serum 25-OH vitamin D, hypogonadism, immobilization and loop diuretic use. Long-term therapy with heparin has been associated with bone loss and vertebral fractures. However, in CHF oral anticoagulants (warfarin) usually are used chronically instead of heparin. Warfarin blocks vitamin K-dependent gamma-carboxylation of osteocalcin and impairs its binding with calcium. Secondary hyperparathyroidism may occur due to impaired renal function and abnormal vitamin D metabolism. Hypogonadotropic hypogonadism appears to be very common in males with CHF. Up to

30% of males with CHF evaluated before transplantation have low levels of testosterone, and this proportion could further increase after cardiac transplantation (Cohen et al., 2003). We have found that trabecular bone loss was related to pretransplantation time of waiting. Also bone resorption markers were increased at this stage reflecting a high bone turnover (Garcia Delgado et al., 2000).

2.1.4 Liver disease

Osteoporosis and osteopenia are frequent complications of chronic liver disease. Its prevalence is high in patients waiting for liver transplant, especially in cholestatic liver disease. In the most important series, densitometric osteoporosis has been described between 31% to 44% (Lopez et al., 1993, Newton et al., 2001, Solerio et al., 2003;Ninkovic et al., 2001; Guichelaar et al.., 2006).

A low bone turnover state has been found in biochemical measurements and histomorphometric analysis (Diamond et al., 1989). Osteocalcin levels are low and correlate with bone formation rate on bone biopsies, and show increases after successful transplantation. However, some reports describe increases in parameters reflecting bone resorption (osteoclast number and bone resorption surface) (Cuthbert et al., 1984).

Reduced bone formation has been related to several toxic factors that could inhibit osteoblast function as excessive alcohol intake or hyperbilirrubinemia. Also a lower IGF-1 sinthesis, that has a direct trophic action upon the osteoblast could be involved. Glucocorticoid used simultaneously reduces bone formation and increases bone resorption. Vitamin D metabolism plays a pivotal role. Patients with end-stage liver disease frequently have low serum levels of 25(OH) vitamin D, since 25-hydroxylation of colecalciferol occurs at the hepatocyte. Fat malabsorption also decreases 25 (OH) vitamin D. In addition, increased vitamin D catabolism, reduced levels of vitamin D-binding protein (DBP), or reduced sunlight exposure can further decrease vitamin D serum levels. However, true osteomalacia is rare in cirrhotic patients (Table 2). Typically, successful liver transplantation reverses most of these factors. Hypogonadism frequently associated in these patients, could be partially responsible for the increased bone resorption.

Reducing bone formation:
-Ethanol excess
-Iron overload (hemochromatosis)
-Hyperbilirrubinemia
-Glucocorticoid use
-Decreased IGF-1
-Decreased 25 (OH) vitamin D_3
Increasing bone formation:
-Hypogonadism
-Glucocorticoid use

Table 2. Involved factors in hepatic osteodystrophy.

In the past two decades significant changes have occurred in the management of chronic liver disease, the waiting time for transplantation and immunosuppressive therapy. Recently an improvement in lumbar spine BMD T-scores pretransplant from -2.5 before 1990 to -1.7 after 1996 has been described (Guichelaar et al., 2006). This data can help to clarify the etiology of bone loss: the severity of liver disease has not changed, the duration of disease before transplantation has been extended and patients can reach older ages. However, nutritional status has improved and bilirubin values decreased. These factors may have contributed to increase BMD before transplantation.

2.1.5 Bone marrow

Bone marrow transplant (BMT) recipients have many known risk factors for developing bone loss: Failure to attain peak bone mass in children and adolescents, hypogonadism, inactivity and induction and consolidation regimens with high dose of chemotherapy, glucocorticoids and irradiation that may damage bone marrow stromal cells and colony-forming unit fibroblast , reducing osteoblastic differentiation. A study in patients before BMT (after chemotherapy) show osteopenia in 24% and osteoporosis in only 4% (Schulte et al., 2000).

2.2 Related to transplantation: Immunossupressor drugs and other factors

2.2.1 Glucocorticoids

Early bone loss has been observed in all solid organ transplants in the first 3 to 6 months, increasing the incidence of osteoporosis and osteopenia (Rodino et al., 1998; Leidig-Bruckner et al., 2001; Eastell et al., 1991). Bone loss primarily affects the spine and proximal femur. Some authors found greater impairment at this level (Keogh et al., 1999; Ninkovic et al., 2002). In patients who already have osteopenia or osteoporosis, this subsequent bone loss can result in a higher number of fractures (Eastell et al.., 1991; Leidig-Bruckner et al., 2001). Traditionally, it has been assumed that high doses of glucocorticoids required for immunosuppression play a major role in this loss. High doses (≥ 1 mg/kg/day) are commonly prescribed immediately after transplantation, with gradual dose reduction over several weeks or months. Total GCs exposure depends on the transplanted organ, number of rejection episodes, and different immunosupressive regimens.

The natural history of post-transplantation osteoporosis suggests that there are two main phases: the early one and the late one. The factors affecting the skeleton differ between this two phases.

The mechanisms associated with bone loss due to glucocorticoid treatment in the first phase are (Table 3):

1) An increase in bone resorption as a result of increased urinary calcium, decrease in intestinal calcium absorption, secondary hyperparathyroidism and hypogonadotropic hypogonadism; 2) Activation of osteoclastogenesis caused by increase of RANKL and decrease of osteoprotegerin (OPG). 3) Corticosteroid treatment decrease the proliferation and function of osteoblasts (by inhibiting the gene expression of osteocalcin, collagen type 1 and IGF-I) and induces its apoptosis (Canalis et al., 2002). **(Fig 1)**

In addition to their direct effects on bone tissue, glucocorticoids can induce severe myopathy, impairing balance and mobility, decreasing weight-bearing activity and increasing fall risk and fractures.

Increase in bone resorption as a result of increased urinary calcium.
Decrease in intestinal calcium absorption.
Secondary hyperparathyroidism.
Hypogonadotropic hypogonadism.
Activation of osteoclastogenesis caused by increase of RANKL and decrease of osteoprotegerine (OPG) levels.
Decrease in proliferation and function of osteoblasts (by inhibiting the gene expression of osteocalcin, collagen type 1 and IGF-I).
Decrease anabolic effects of TGF-beta.
Induces Osteoblast apoptosis.

Table 3. Effects of high doses of glucocorticoids in bone loss.

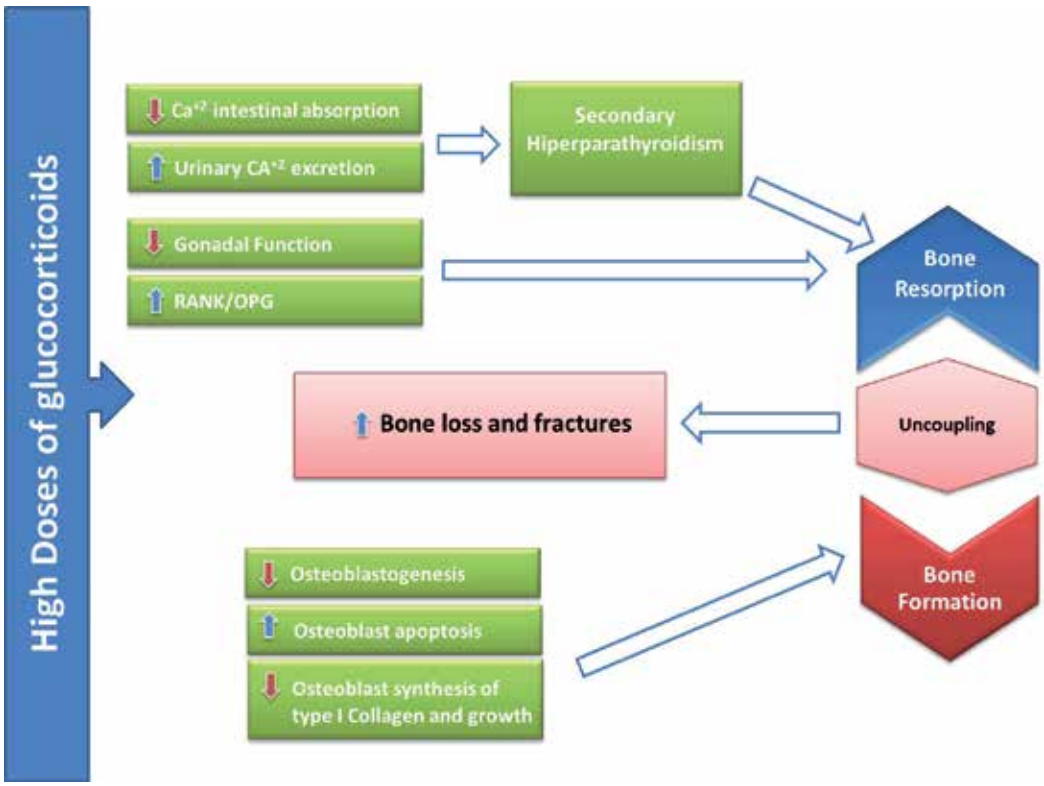

Fig. 1. Effect of high doses of glucocorticoids in bone loss

Effect of glucocorticoids in the WNT pathway: The Wnt pathway and the Bone Morphogenetic Protein (BMP) seem to be involved in the pathogenesis of glucocorticoid-induced osteoporosis, suppressing osteoblast differentiation and activity. BMP and Wnt pathway are regulated by several mechanisms: one of them are proteins that act as extracellular antagonists of BMP (Noggin, Chordin, Twisted gastrulation, Grelin, Sclerostin,

Follistatin and Dan), while others act as Wnt antagonist: Frizzled-related protein (sFRP), Dickkopf (Dkk) and Cerberus. Glucocorticoids can affect these signaling pathways, but the exact mechanisms are not been clarified. Recent studies suggest that glucocorticoids induce an alteration in osteoblast function by increasing Wnt antagonists with the subsequent suppression of this pathway. Recent reseach has shown that dexametasone, increases follistatin and DAN (BMP antagonists), sFRP-1(Wnt antagonist) and axin-2 (inhibitor of Wnt signal) . Simultaneously, alendronate and PTH (1-34), which have demostrated to be effective in the treatment of steroid osteoporosis, were able to antagonize the increase in this proteins induced by dexametasone (Hayashi et al., 2008).

The potential impact of glucocorticoid dose as a determinant of bone loss is supported by the absence of bone loss at the lumbar spine and proximal femur in renal transplant patients treated with low doses of steroids and tacrolimus (Goffin et al., 2001). We have previously reported that steroid withdrawal in patients who have undergone a successful liver transplant accelerates the recovery of lumbar spine bone density without adverse effects on graft tolerance (Martínez Díaz-Guerra et al., 2002).

Moreover, higher rates of fracture occurring after cardiac (Shane et al., 1996) and lung (Shane et al., 1999) transplantation, in which higher doses of steroids are use, would be consistent with their role in the pathogenesis of post transplant osteoporosis.

The late phase observed in the postrasplant period takes place when the glucocorticoid doses are usually tapered below 5 mg per day. Then, osteoblast function recovers and consequently, an increase in bone formation and recoupling of bone remodeling activity is observed. During this later phase, rates of bone loss slow and there may even be some recovery, particularly in cancellous bone (Kulak et al., 2006). It is also found that despite an initial decrease in post transplant BMD, biopsies in the iliac crest showed improvement in histomorphometric parameters 4 months later (Guichelaar et al., 2003).

In conclusion, current evidence suggests that bone loss after transplantation is caused by an initial increase in bone turnover and resorption, plus a decrease in bone formation. Later, increases in bone formation could overcome resorption. These changes would be consistent with the rapid decrease in BMD observed in the first months after transplantation and recovery to baseline values, as most of studies show.

2.2.2 Other immunosuppressive drugs

The effect of these drugs in humans is difficult to study because they are used in conditions that by themselves affect bone remodeling, and they are rarely used in monotherapy so, the potential deleterious effect of one single agent could not be ascertained.

The role of calcineurin inhibitors in post-transplant bone loss is unknown. Tacrolimus is a calcineurin inhibitor that suppresses T cell activation and the production and release of IL-2 and other cytokines. It induces severe trabecular bone loss in rats, but this effect appears to be less severe in humans (Epstein, 1996). Cyclosporin A (CyA), another calcineurin inhibitor, also appears to have adverse effects in mouse models, inducing high turnover and reducing bone mass. Some studies in humans suggest a similar effect in patients with liver (Giannini et al., 2000), and cardiac (Thiebaud et al., 1996) transplantation. In a research of 360 patients with liver transplantation due to chronic cholestatic liver disease, the post transplant bone gain was lower, and the number of fractures was higher in patients treated with CyA than in those receiving tacrolimus (Guichelaar et al., 2007). Other authors have found greater

fracture incidence in patiens receiving CyA treatment than in those treated with tacrolimus (Monegal et al., 2001).
In other liver transplanted recipients study, although bone mass losses were similar in patients on CyA regimen than in those on tacrolimus, histomorphometric changes after transplantation were different between groups. Patients treated with tacrolimus had an improvement in trabecular bone architecture compared with patients receiving CyA (Guichelaar et al., 2004). These findings suggest that patients treated with tacrolimus may have faster recovery of bone metabolism after the initial phase of bone loss compared with those treated with CyA.
A study comparing CsA monotherapy versus prednisone and azathioprine regimen in renal transplant recipients did not found any differences in bone loss or bone histomorphometric parameters (Cueto-Manzano et al., 2003). Furthermore, a major side-effect of CsA therapy is dose-related nephrotoxicity, often leading to secondary hyperparathyroidism, which may also adversely affect bone health.
Other immunosupresive agents such as Mycophenolate mofetil (104), rapamycin or azathioprine have shown no effects on bone in murine models (Maalouf et al., 2005).

3. Bone loss and fractures after transplantation

The majority of longitudinal studies show a decrease in bone mineral density at the lumbar spine and hip that occurs in the first year after solid organ transplantation. The amount of bone loss ranges between 3% and 10%, particularly in the first 3-6 months. Rapid bone loss and major involvement of lumbar spine (trabecular bone tissue) are findings probably related to the large doses of glucocorticoids used immediately after transplantation. Rates of lumbar spine bone loss slow thereafter, with stabilization by 6–12 months and even some recovery after liver, lung, and heart transplantation. However, most studies do not document recovery of bone mass at the hip. BMD changes after renal transplantation are different since continued bone loss after the rapid initial bone loss may be observed. Prevalence of densitometric osteoporosis is quite variable depending on type of organ transplantation and time elapsed since transplantation (Hawkins et al., 1994).
With regard to fractures, a high incidence of them (between 20% and 40% in most studies) has been documented. In heart and liver transplant recipients, the incidence of new fractures parallels the timing of the most rapid bone loss, with most fractures occurring within the first year after transplantation (Eastell R, 1991; Henderson et al., 1995; Shane et al., 1996; Ramsey-Goldman et al., 1999).
Fractures usually affect the spine and ribs in liver, cardiac, or lung recipients, whereas long bones are more easily fractured in renal transplant recipients. However, fracture incidence may have decreased in the last years. This is probably related to the wide implementation of immunosuppressive regimens that use lower doses of glucocorticoids and for a shorter period of time. Indeed, bone loss and fractures remain unacceptably high in several recent studies.
Risk factors for fractures after transplantation include older age, prevalent fractures before transplantation, postmenopausal status, and lower body mass index. Additional risk factors in renal transplant recipients include the presence of diabetes mellitus and prolonged dialysis. The predictive roles of pretransplantation BMD and cumulative glucocorticoid dose are controversial. Associations between these risk factors and bone loss or fractures are

not consistent across studies. Even patients with normal pre-transplant BMD may suffer fracture after transplantation. Therefore, it is usually not possible with current clinical tools to predict whether individual transplant recipients will fracture after transplantation.
In a nested case-control study of transplant recipients (kidney, liver, lung, heart), multivariate analysis showed that post-transplant fracture rate was highest among those with a history of hyperthyroidism, pretransplant diabetes, fracture, or corticosteroid use, and among those currently exposed to antidepressants, narcotics, sirolimus, and loop diuretics (Shane et al., 2009 uptodate). Use of bisphosphonates or calcitonin was also a predictor of fracture, likely indicating the presence of pre-transplant osteoporosis.
In some studies, the rate of post-transplant fracture is decreasing, in part due to increased recognition of the problem, which has resulted in changes in immunosuppressive regimens (reduction in dose and duration of glucocorticoids) and earlier identification and treatment for osteoporosis (Shane et al., 2004; Compston et al., 2003)

3.1 Kidney transplantation

Rates of bone loss are greatest in the first 3-18 months and range from 4-9% at the spine and 5-8% at the hip.
After renal transplantation fractures affect appendicular sites (hips, long bones, ankles, feet) more commonly than axial sites (spine and ribs). The majority of fractures occur within the first 3 years. Fracture prevalence varies from 7-11% in nondiabetic renal transplant recipients, but is considerably higher in patient transplanted because of diabetic nephropathy and in those who receive kidney-pancreas transplants. (Nowacka-Cieciura et al., 2006)
With regard to the difference in the prevalence of fracture in end stage renal disease patients referred to kidney transplant or those who continued dialysis, a large study realized with 101,039 patients with end stage renal disease demonstrated that kidney transplantation was associated with a 34% greater risk of hip fracture than continued dialysis (Nisbeth et al., 1994).

3.2 Lung transplantation

During the first year after lung transplantation, rates of bone loss at the lumbar spine and femoral neck range from 2 to 5%. In another study conducted with 70 patients awaiting lung transplantation the prevalence of vertebral fractures was 29% in patients with chronic obstructive pulmonary disease and 25% in patients with cystic fibrosis (Shane et al., 1996). Fracture rates are also high during the first year after lung transplantation, ranging from 18 to 37%.

3.3 Cardiac transplantation

The most rapid rate of bone loss occurs in the first year. Spinal BMD declines by 6-10% during the first 6 months, whereas femoral neck BMD falls by 6-11% in the first year. The rate of bone loss slows after the first year and spine BMD may increase after the third year (Cohen et al., 2003). Densitometric osteoporosis at the lumbar spine and femoral neck has been reported in approximately 28% and 20% respectively of long term transplant patients (Chou et al., 2006).
Vertebral fracture incidence ranges from 20-36% during the first 1 to 3 years after cardiac transplantation. One prospective study shows that 36 percent of all patients and 54 percent

of women sustained a fracture after this procedure, 85 percent of which occurred within the first six months (Shane et al., 1996). Women with the lowest pretransplant hip BMD were at highest risk of fracture. In men, pretransplant BMD did not differ between those who went on to fracture and those who did not.

3.4 Liver transplantation

Spine BMD declines by 2-24% during the first year in earlier studies, particularly during the first 3-6 months. Some authors report higher rates of bone loss and fracture in patients who have alcoholic cirrhosis, primary biliary cirrhosis, and primary sclerosing cholangitis (Lopez 1992). Rates of bone loss have been lower in more recent studies. In the second year after transplantation, lumbar BMD recovered or even exceeded baseline levels (Guichelaar et al., 2006). Although early studies showed a predominance of post-transplant bone loss and fractures in the lumbar spine (Compston et al., 2003), more recent studies reported higher bone loss at the hip (Keogh et al., 1999; Ninkovic et al., 2002; Crawford et al., 2006). It seems that there are differences in the natural evolution of lumbar and femoral BMD, with greater loss of femoral bone that persists after the first year after transplantation. As an example, after 3 years, BMD at the femoral neck improved, but still remained below baseline levels (Monegal A, 2001). Other studies found a decrease in BMD at the femoral neck at 6 and 12 months, even despite treatment with bisphosphonates, suggesting a lesser effect of these drugs at cortical bone (Keogh et al., 1999), (Ninkovic et al., 2002), (Monegal et al., 2009).
Fracture rates after liver transplantation are highest in the first 6-12 months. Range from 24 to 65% and the ribs and spine are the most common sites. Women with primary biliary cirrhosis and the most severe preexisting bone disease appear to be at greatest risk. In a study of 37 patients receiving liver transplantation between 1993 and 1995, an incidence of 27% of vertebral fractures in the first three months after transplantation was found (Ninkovic et al., 2000). A subsequent study of the same group, done between 1995 and 1998, showed that this incidence was only 5%. Between both studies there was a considerable reduction in the dose and duration of glucocorticoid treatment, although the use of cyclosporine and tacrolimus barely changed (Ninkovic et al., 2002).

3.5 Bone marrow transplantation

The pattern of bone loss after bone marrow transplantation (BMT) is different from other forms of osteoporosis, being more persistent and severe in cortical bones, such as femoral neck than in trabecular bones, such as lumbar spine (Hatutman et al., 2011)). Bone marrow transplant (BMT) recipients have many known risk factors for developing decreased bone mineral density after transplantation. The pathogenesis of bone disease following BMT differs from others form of post-transplantation osteoporosis; recipients are usually younger and the time from the diagnosis to the BMT does not exceed 2 years; history of prolonged bed rest is uncommon. Immunosuppressive drugs are used in relatively low doses and for short periods of time (Ebeling et al., 1999). Glucocorticoid use is restricted to the treatment of graft-versus- host disease (GVHD).
Lumbar spine BMD declines by 2-9% and femoral neck BMD falls 6-11% during the first year following transplantation. Lumbar spine BMD begins to recover after 12 months, returning to baseline levels at 48 months. The extent of recovery at the femoral neck is less (Schulte et al., 2004). The presence of chronic GVHD is another factor associated with higher risk of osteoporosis in these patients. Avascular necrosis develops in 10-20% of allogenic

BMT patients; its development may be facilitated by a deficit in bone marrow stromal stem cell regeneration and low osteoblast number.

4. PTH. 25-OH-D. Bone remodeling in postransplantation bone disease

4.1 PTH

Elevated PTH levels have an adverse effect on bone health increasing turnover and decreasing bone mass, especially in cortical bone.

Some studies reflect a slight increase in PTH levels in the first month after transplantation. It could be related to vitamin D deficiency, calcium malabsorption or decreased tubular reabsorption of calcium, consequence of steroid treatment (Compston et al., 1996). PTH levels may remain elevated in the long-term due to chronic renal failure induced by cyclosporin (Floreani A, 2001; Crosbie et al., 1999).

4.2 25-OH vitamin D

Inadequate levels of vitamin D have been described in patients with end-stage liver diseases prior to liver transplantation, and this may play a role in the aetiology of lower mineralization after transplantation. Our group found that 91% of liver transplanted patients had insufficient serum levels of 25(OH)D at transplantation time. After adequate supplementation, serum 25(OH)D levels increased from 3 months onwards (Guadalix et al., 2011). A positive correlation between serum 25(OH)D levels at 3 months and BMD increase at 6 months was found suggesting that this vitamin has a positive effect on mineralization (Crosbie et al., 1999). In our study serum 25(OH)D levels showed positive correlation with the percentage change in total hip and femoral neck BMD at 12 months of treatment (Guadalix et al., 2011). These results suggest that vitamin D could have a main role in bone loss prevention after liver transplantation.

4.3 Bone remodeling in postransplantation bone disease

Bone turnover markers can provide information about the mechanisms of bone loss in post-transplant period.Our group has previously reported an increase in bone turnover markers such as osteocalcin after liver transplantation (Valero et al., 1995; Hawkins et al., 1994). No significant changes in urinary hydroxyproline, one and two years after transplant were found; however urinary excretion of NTX (amino-terminal telopeptide of collagen type I) showed a significant decrease after two years compared with baseline values(Giannini et al., 2000), while other found that values of deoxypyridinoline doubled compared to baseline, two months after transplantation (Crosbie et al., 1999). We also found that β-CTX decreased as from 3 months both in patients on bisphosphonate treatment as in patients receiving only calcium plus vitamin D, reflecting a reduction of bone resorption after liver transplantation (Guadalix et al., 2011). Greater reductions in β-CTX may be obtained with intravenuous bisphosphonates. A significant decrease in urinary deoxypyridinoline in heart transplant recipienters after intravenous pamidronate traeatment (Shane et al., 1998) and in β-CTX levels 6 months after liver transplanted in 13 patients treated with intravenous zoledrónico acid (Misof et al., 2008) was found. Other investigators found an early increase (one and 3 months after heart transplantation) in resorption markers hydroxyproline, pyridonoline and desoxypyridinoline and a decreased in osteocalcin, recovering all baseline values at 6 months (Shane et al., 1997). Several studies have investigated the OPG / RANKL in the post-transplant period. Results are

not homogeneous. High levels of both, OPG and RANKL in the first 14 days after liver transplantation compared to the control group were found (Fabrega et al., 2006). In the other hand, serum OPG in 57 patients at 3 and 6 months after cardiac transplantation wa correlated with bone loss at the lumbar spine and femoral neck sites, after 6 months. Serum OPG alone accounted for 67% of the variance of lumbar spine bone density changes over the first 6 months post transplantation leading to the conclussion that serum OPG levels decline consistently in all patients following initiation of immunosuppressive therapy and are independently correlated with changes in bone density (Fahrleitner et al., 2003).In another study in patients with kidney transplant, levels of OPG and RANKL did not differ between healthy volunteers and transplant patients (Malyszko et al., 2003).

5. Gonadal status and postransplatation bone disease

Hypogonadotropic hypogonadism is frequently found both before and after transplantation and may play a role in the multifactorial pathogenesis of immunosuppression-induced bone loss. Sex-steroid deficiency in either sex results in an increase in bone turnover with an imbalance in bone formation and bone resorption. Few studies have assessed the status of gonadal function after transplantation and its relationship with bone mass. Many premenopausal women and men undergoing solid organ transplantation have temporary hypogonadism, most often related to the effects of glucocorticoids and chronic illnesses (Fleischer et al., 2008; Tauchmanovà et al., 2005). In some cases (i.e. chemotherapy and/or radiation therapy for hematopoietic stem cell transplantation), hypogonadism is permanent (Tauchmanovà et al., 2003). In men and women undergoing transplantation, testosterone and estrogen-progestin replacement, respectively, have been shown to slow bone loss (Isoniemi et al., 2001; Kananen et al., 2005). Hypogonadism is a common finding among patients with terminal liver disease, especially in males. Incidence was estimated up to 70% (Guichelaar et al., 2004). There are few studies about change in sex hormones after liver transplantation, some authors have reported an increase in free testosterone, although the recovery of normal levels has not been achieved in all patients (Floreani et al., 2001; Monegal et al., 2001). In a study of 10 liver transplant recipients followed for 12 months after transplantation, before transplantation, 90% of patients had a decrease in testosterone levels and reported decreased libido and erectile dysfunction .After transplant, total testosterone levels had doubled, and free testosterone increased tenfold. Patients reported early improvement in sexual function (6 to 8 weeks after transplantation). It was suggested that pretransplant abnormalities in gonadal function are mainly due to liver failure and are reversible in most patients (Madersbacher et al., 1996). In premenopausal women, normal menses usually resumes after liver transplantation (Mass et al., 1996).

Low levels of testosterone are quite common early after cardiac transplantation, and may be found in up to 50% of men (Guo et al., 1998). In addition, a significant relationship between low levels of serum testosterone and rates of femoral neck bone loss during the first 6 month after transplantation have been found by some authors (Shane et al., 1997). Fleischer et al., (2008) studied 108 male heart transplant patients. One month after transplantation, total testosterone levels were below normal in 63% of them while 33% had decreased levels of free testosterone. 15% of patients had elevated gonadotropin a month after transplantation, increasing to 29% at 6 months. These data suggest a suppression of the hypothalamic-pituitary-gonadal axis immediately after transplantation, with subsequent recovery. Authors attributed this to steroid therapy. Prednisone dose was found to be the main

determinant of the values of total and free testosterone). No relationship was found between post-transplant bone loss and testosterone levels, probably because patients were treated with calcitriol or alendronate.
In most studies, testosterone levels return to normal by 6 to 12 months after transplantation (Sambrook et al., 1994) but up to 20% of patients receiving prednisone and cyclosporine A may persist with low serum total testosterone levels 3 years after cardiac transplantation (Stempfle et al., 1999).
Regarding the role of other immunosuppressive agents on gonadal function cyclosporine A decreases testosterone by affecting both the hypothalamic-pituitary- gonadal axis (Sikka et al., 1988) and by direct inhibition of testicular synthesis of testosterone in murine models (Seethalakshmi et al., 1990). However, cyclosporine did not seem to affect testosterone levels in humans (Fleisher et al., 2008; Samojlik et al., 1992).
Some authors recommend hormonal treatment in post transplant osteoporosis in men and premenopausal women with hypogonadism, if there are no contraindications (Shane et al., 2009 up-to-date). Androgen replacement therapy has shown skeletal benefit (increase in BMD) only in men with hypogonadism. It has been demonstrated in an uncontrolled study of postmenopausal liver transplantation recipients, that the use of transdermal estradiol was effective in increasing BMD of lumbar spine and femoral neck over a period of two years (Isoniemi et al., 2001).

6. Prevention and treatment of osteoporosis

6.1 General measures before transplantation

The same measures used to prevent osteoporosis in the general population apply to transplant recipients, regardless of the pretransplant BMD measurement. It is recomended that all candidates for organ transplantation follow a throughfully evaluation in order to identificate and correct risk factors and to implement measures to improve bone health (Table 4).

Before Transplantation	After transplantation
- Measurement lumbar spine and hip BMD. If BMD is low, it should be evaluated secondary causes of osteoporosis. - Patients with kidney failure should be avaluated and treated for renal osteodystrophy. - Perform spine radiograph to detect prevalent fractures. - Recommend appropriate intake of calcium (1000-mg/day) and vitamin D (800 IU). - Patients with osteoporosis should be evaluated and treated according to general guidelines.	- Consider initiating preventive therapy in most patient (even those with normal BMD): calcium, vitamin D and antiresorptive agents. - Perform annual BMD measurement. - Perform annual Spine Radiograph. - Annual measurement of bone turnover markers.

Table 4. General recommendations for prevention and treatment of osteoporosis

Lifestyle factors, such as immobilization, smoking, and alcohol abuse, should be avoided. Concomitant use of medications that can negatively impact skeletal health should be minimized whenever possible. Hypogonadism should be sought and corrected, particularly in males, where symptoms are easily confounded with those of preexisting chronic disease or adverse effects of concomitant medication. All patients should receive the recommended daily allowance for calcium (1000-15000 mg/day) and vitamin D (800 IU/day). Higher vitamin D doses should be given if the patient is vitamin D deficient (serum 25-hydroxyvitamin D level >20 ng/ml [50 nmol/L]). Although calcium and vitamin D do not prevent transplantation-related bone loss, randomized trials assessing antiresorptive therapy, such as bisphosphonates, have been carried out in the setting of concomitant calcium and vitamin D repletion.

Prevention of early bone loss after transplantion have been reported with specific resistance training programs (Mitchell et al., 2003). Regular weight-bearing exercise (30 minutes, three times per week) has proved also to be beneficial for the prevention and treatment of osteoporosis. Because of the high prevalence of osteopenia and osteoporosis in patients awaiting transplantation and the morbidity caused by osteoporosis after transplantation, it is recommended that candidates for organ transplantation undergo measurements of BMD of the hip and spine, preferably at the time of acceptance to the waiting list. Low BMD before transplantation has been pointed out as a risk factor for fractures after transplantation. In addition, spine radiographs should be performed to detect prevalent fractures .Patients who have a history of fracture or have osteoporosis on DXA (T-score ≤ -2.5) before transplantation should be evaluated for secondary causes. When a secondary cause (i.e. hypogonadism) is identified, appropriate treatment is recommended prior to transplant. In addition to the treatment (when possible) of secondary causes, some patients may benefit from additional osteoporosis therapy, such as bisphosphonates, while awaiting transplant. Patients with osteopenia should be considered for prevention (calcium, vitamin D and/or antirresorptives) evaluating risk factors. Alternatively, patients with normal BMD can defer medical therapy until immediately after transplantation. For patients with end-stage renal disease, an evaluation and treatment for renal osteodystrophy according to accepted guidelines is highly recommended.

6.2 Therapeutic measures of post-transplantion bone loss

Several drugs have been studied for the treatment of osteoporosis after transplantation. Many of these studies were done with small number of patients, were not randomized or not compared with control group. Also, patients were not selected based on T-score or risk factor (beyond the transplant). There is no consensus on candidates for the treatment or the drug of choice.**G**iven the accelerated bone loss that occurs immediately after transplantation many experts recommend preventive treatment for all patients receiving solid organ transplantation, regardless of pretransplant BMD (Maalouf NM, 2005; Cohen et al., 2006). This approach is based on observational data that show an overlap in BMD values between the pre-transplant patients with posttransplant fracture and those without fractures (Leidig-Bruckner et al., 2001; Shane et al., 1996). The lack of reliable clinical predictors to identify individual patients who will experience osteoporotic fractures renders all transplant recipients candidates for preventive therapy. Treatment should be started immediately after transplantation.

Since lumbar BMD starts to recover in many patients at 12 months after transplantation, long-term treatment may be unnecessary (Cohen et al., 2006). Duration of treatment

depends on patient characteristics, such as time of steroid therapy withdrawal, presence of other risk factors for low bone mineral density and fractures as well as information provided by the measurement of BMD.
Another approach to the management of transplanted patients is to apply similar guidelines as those used for the prevention of glucocorticoid-induced osteoporosis. There are several guidelines for the prevention of glucocorticoid-induced osteoporosis. Collectively, they suggest preventive therapy for patients with clinical risk factors for osteoporosis and fracture (age ≥65 years, previous fragility fracture) or in patients without other risk factors if BMD T-score is below -1.0 or -1.5 (Shane E, 2009).

6.2.1 Bisphosphonates

These potent antiresorptive agents are an obvious option in preventing the rapid bone loss, that occurs mainly in the early phase after transplantation. Bisphosphonates are considered the medical therapy of choice for the prevention of transplantation-related bone loss. Although there are conflicting data both oral and intravenous bisphosphonates appear to be effective in these patients.
Below shows some of the results obtained with bisphosphonates treatment in different types of transplants.
In a study of 99 subjects receiving stem cell transplantation, patients were randomly assigned to receive calcium and vitamin D or calcium and vitamin D plus pamidronate (60 mg intravenously six times over the first post-transplant year). Treatment with pamidronate prevented spine bone loss (0 percent in pamidronate group versus -2.9 percent in calcium group), and reduce hip bone loss (-5.5 percent and -7.8 percent in the pamidronate and calcium-vitamin D groups, respectively) (Kananen et al., 2005).
In a trial of 62 adults undergoing liver transplantation, patients were randomly assigned to receive infusions of zoledronic acid (4 mg) or placebo within seven days of transplantation. BMD was measured 3, 6, 9, and 12 months later. Zoledronic acid group lost significantly less bone at the hip at all time points (Crawford BA, 2006). In the spine, the zoledronic acid group lost less bone at three months, but the difference between the two groups was no longer significant at 12 months because of improvements between 3 and 12 months in the placebo group. Zoledronic acid sometimes caused postinfusion hypocalcemia and temporary secondary hyperparathyroidism.
Oral bisphosphonates are also effective in preventing bone loss after transplantation (Shane et al., 2004; Atamaz et al., 2006). As an example, in a trial of 98 subjects receiving a liver transplant, subjects randomly assigned to alendronate (70 mg weekly) versus no alendronate had significant increases in lumbar spine (5.1and 8.9 percent) and femoral neck (4.3 and 8.7 percent) BMD at 12 and 24 months, respectively, compared with the control group (Atamaz et al., 2006). All subjects received calcium (1000 mg daily) and calcitriol (0.5 mcg daily).
Our group studied the effect of risedronate in liver transplant patient. The main findings of our study are that liver transplanted patients with low bone mineral density who receive either Risendronate combined with calcium and vitamin D3 or vitamin D3 and calcium alone showed a significant increase in spine BMD at 12 months compared to baseline values. In addition, risedronate patients showed a significant increase in intertrochanteric BMD, but we were not able to find any significant differences between groups. Hence, these results suggest that weekly risedronate after liver transplantation combined with 1000 mg/day of calcium and 800 IU/day of vitamin D are not superior to the administration of calcium and vitamin D alone (Guadalix S, 2011). Significant

improvement in BMD at the lumbar spine was also observed 12 months after liver transploant in the control group. This response may be related to the administration of calcium and vitamin D3 itself, but also to improvement in general health, mobility, muscle mass, and nutrition as a consequence of better liver function.
A recent meta-analysis in 364 liver transplant patients from 6 randomized controlled trials have found that bisphosphonate therapy improved lumbar spine BMD by 0.03 g/cm^2 (95% CI 0.01-0.05 g/cm^2, p=0.02) at 12 months post-liver transplantation compared to the control group. However, a statistically significant change in femoral neck BMD could not be demonstrated in this meta-analysis. Data on fractures could not be analyzed (Kasturi et al., 2010). In a study of 34 lung transplant recipients (with cystic fibrosis antecedents), pamidronate therapy versus calcium and vitamin D showed a significant increase in bone mass at 2 years in lumbar spine and total femur. There was no difference in fracture incidence (Aris et al., 2000).
In patients after allogenic stem cell transplantation pamidronate reduced bone loss at the spine, femoral neck and hip by 5.6, 7.7 and 4.9% respectively after 12 month of treatment. However, at 24 month, only differences at BMD of total hip remained statistically significant between study groups (Grigg et al., 2006). Other study in 12 patients treated with zoledronic acid show that 12 month after infusion, total hip BMD increased in 75% of the patients and femoral neck BMD increased in 11 of 12 patients. Spinal BMD only increase in four (D'Souza et al., 2006).
Based upon the above trials, we suggest bisphosphonates as first choice for prevention of transplantation-related bone loss. There are few data to support the use of one bisphosphonate over another. Many of the trials used intravenous bisphosphonates due to ease of administration, especially in post-transplant patients who are required to take many oral medications. There are no trials comparing oral to intravenous bisphosphonates in the immediate post-transplant setting. The decision should be based upon individual patient preferences and abilities. The safety and efficacy of bisphosphonates for prevention of transplantation-related bone loss in patients with chronic kidney disease has not been carefully evaluated, and in general, there is limited data on the level of renal impairment at which bisphosphonate use should be avoided and whether this level is the same for iv bisphosphonates. In the majority of trials, individuals with serum creatinine concentrations above the upper limits of normal were excluded from participation. Despite these concerns, however, it is usually recommend their use after renal transplantation, at least during the first year when rates of bone loss are most rapid.

6.3 Other therapies

Replacement doses of calcium and vitamin D (400-1000 IU/day) do not prevent clinically significant bone loss after transplantation, but active metabolites of vitamin D could reduce post-transplantation bone loss, probably by reversing glucocorticoid-induced decreases in intestinal calcium absorption.

6.3.1 Calcidiol (25-hydroxivitamin D) and alfacalcidol (1α-hydroxivitamin D)

In renal transplant recipients calcidiol (40 μgr/day) prevents spine and femoral bone loss and is associated with a significant decrease in vertebral deformities (Talalaj et al., 1996). Consistently with these findings, our group have found that in patients randomized immediately after cardiac transplantation to 32000 IU/week of oral calcidiol for 18 months,

lumbar spine BMD increased ~5%, whereas those who received cyclical etidronate or nasal calcitonin, showed decreases in spine BMD (Garcia-Delgado et al., 1997). Also, alfacalcidol therapy has been associated with an increase in BMD or prevention of additional bone loss in renal (El-Agroudy et al., 2003) and cardiac recipients (Van Cleemput et al., 1996).

6.3.2 Calcitriol

Calcitriol may suppress bone resorption indirectly by facilitating intestinal calcium absorption and suppressing PTH secretion. Studies of calcitriol alone and those that compare calcitriol and bisphosphonates suggest that calcitriol may also prevent early post-transplant bone loss, particularly at the proximal femur. Positive effects on BMD have been found with higher doses of calcitriol (> 0.50 μgr/day) in heart, lung or renal transplantation, whereas lower doses (0.25 μgr/day) are relatively ineffective. Use of calcitriol requires close monitoring of serum and 24 hour urine calcium, because it is associated with hypercalcemia and hypercalciuria in >50% patients.In a study of 65 patients undergoing cardiac or single lung transplantation, patients were randomly allocated to receive placebo or calcitriol (0.5-0.75 microg/day), the latter for either 12 months or 24 months. Bone loss at the proximal femur was significantly reduced or prevented by treatment with calcitriol for 2 years compared with treatment with calcium alone (Sambrook et al., 2000).
Other randomized study compared the efficacy of 6 months treatment with either calcitriol (0.5 microg/day) or two cycles of etidronate plus calcium in preventing bone loss in 41 patients undergoing cardiac or lung transplantation. Compared with an untreated reference group, both therapies offered significant protection at 6 months in lumbar spine and etidronate provided significant protective carryover after therapy had been discontinued (Henderson et al., 2001).Although calcitriol appears to be effective in preventing bone loss after transplantation (Sambrook et al., 2000; Shane E, 2004) it should not be selected as first-line treatment because of their limited effectiveness and narrow therapeutic window.

6.3.3 Calcitonin

Although calcitonin is effective in preventing bone loss in postmenopausal women, it has not been shown to be superior to calcium in transplant recipients (Välimäki et al., 1999). In one trial, the combination of continuos oral calcitriol (0.5 microg/day) and nasal salmon calcitonin (200 U/day) for the first 3 months was inferior to intravenous pamidronate (0.5 mg/kg body weight) every third month in attenuating bone loss three months after cardiac transplant but similar at 18 months in 26 cardiac transplant recipients (Bianda et al., 2000).

6.3.4 Recombinant parathyroid hormone (PTH)

PTH 1-34 (teriparatide) has been shown to improve BMD in patients with glucocorticoid-induced osteoporosis, but there are few studies evaluating PTH for the prevention of post-transplant osteoporosis. It a small trial 24 kidney recipients patients were treated with 20 μg of teriparatide/daily/6 months, it was shown that femoral neck BMD was stable compared to the placebo group. Lumbar spine BDM and radial BMD, histomorphometric bone volume and bone matrix mineralization status remained unchanged in both groups. (Cejka et al., 2008). Recombinant parathyroid hormone (PTH) has not been well studied in this population. Patients who have received total body irradiation during hematopoietic stem cell transplantation or who have primary or secondary elevations in PTH are not candidates for recombinant PTH therapy.

6.3.5 New therapies

Promising new agents for transplantation osteoporosis include new potent anticatabolic drugs such as human antibodies to receptor activator of nuclear factor kb-ligand (RANKL) (denosumab), and catepsin k inhibitors.

6.4 Monitoring

There is no consensus on the optimal strategy for monitoring patients on therapy. Patients on antiresorptive therapy are measured BMD every year after transplantation. Patients with normal BMD can be follow up with DXA every two years, depending also of other risk factors . In patients who require continuous treatment with glucocorticoids (prednisone ≥ 5 mg / day), BMD measurement is recommended annually. An effort should be made to find the lowest prednisone dose compatible with graft survival.

7. Summary and conclusions

Although bone loss and fractures after transplantation seem to be lower than those reported years ago, they remain being a main long term postransplant complication. An effective approach should incorporate pre-transplant measures to detect and to treat preexisting bone diseases. Oral or intravenous bisphosphonates, in conjunction with calcium and vitamin D, are effective in preventing post-transplantation bone loss when started shortly after grafting. The optimal dose, timing, and frequency, particularly of intravenous bisphosphonate administration, remain to be determined. At present, most controlled trials lack sufficient statistical power to demonstrate efficacy for fracture prevention, so treatment regimens are based on results of effects on the surrogate end-points, bone densitometry, and bone turnover markers. More studies are required to determine the best agent and route of administration to prevent this common complication of organ transplantation. The future challenge is to achieve adequate immunosuppression without corticosteroids, with drugs not damaging bone. This approach, together with improved bone health before the transplant would be an effective strategy to reduce post-transplant osteoporosis.

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17

The Skeleton Abnormalities in Patients with Neurofibromatosis Type 1: Important Consequences of Abnormal Gene Function

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1. Introduction

Neurofibromatosis type 1 [Nf-1, OMIM #162200[1]], formerly known as von Recklinghausen disease, is one of the most frequent disorders affecting mankind, inherited as an autosomal dominant trait. The relatively high prevalence, with an incidence at birth of approximately one in 2500 to 3500 live births, and a progressive nature of the disorder, notable for its phenotypic variability with almost 100% penetrance, as well as high proportion of sporadic cases (almost 50% of de novo mutations), constitute for the clinical magnitude of this disease. Multiple café au lait spots [CALs], axillary and inguinal freckling, multiple discrete cutaneous neurofibromas [NFM] and more prominent plexiform neurofibromas [PNF], and iris Lisch nodules constituted for the cardinal signs of the disease. Learning disabilities and attention deficits states, but usually with normal intelligence in adulthood, are present in at least 50% of individuals with Nf-1. Neurofibromatosis type 1 belongs to the group of disorders with significantly increased risk of tumorigenesis. The other significant manifestations of Nf-1 include bone dysplasias, clinically presented as progressive dystrophic scoliosis, vertebral dysplasia, overgrowth and tibial dysplasia with pseudarthrosis, and vasculopathy. Pubertal development is usually normal, but precocious puberty, especially in those with an optic chiasm glioma, as well as delayed puberty, may commonly occur in children with Nf-1. The life expectancy of Nf-1 patients is assumed to be reduced by 15 years. The most important causes of early death in these patients are malignant peripheral nerve sheath tumors and severe complications of vasculopathy (Friedman et al., 1999; Jett & Friedman, 2010; Larizza et al., 2009).
Despite the possibility of molecular testing, the diagnosis of Nf-1 is still based on clinical findings and is usually unequivocal in all but the young children (DeBella et al., 2000b). The diagnostic criteria for Nf-1 (Tabl. 1) were developed by the US National Institutes of Health (National Institute of Health [NIH], 1988) and are generally accepted worldwide for routine clinical use (Ferner et al., 2007; Williams et al., 2009). The disease is characterizes by extreme clinical variability, not only between unrelated, but also among affected individuals within a

[1] Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. MIM Number: #162200, 07/06/2011. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

single family carrying the same type of mutation, and even within a single person with Nf-1 at different times in life. Penetrance of *NF1* gene mutation is virtually complete (100% of penetration) after childhood and the frequency of more serious complications increases with age. Various manifestations of Nf-1 have different characteristic times of appearance (DeBella et al., 2000b; Boulanger & Larbrisseau, 2005; Friedman ed., 1999; Williams et al., 2009). The clinical NIH diagnostic criteria are both highly specific and highly sensitive only in adults. Less than half of youngest children with no family history of Nf-1 meet NIH criteria, although almost all do by adolescence. Yet, neonates who inherited *NF1* mutation from one of the parents can usually be identified within the first year of life by the presence of numerous CALs (DeBella et al., 2000b).

The presence of two or more of the following features is required for the diagnosis of Nf-1:
1. Six or more café au lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
2. Two or more neurofibromas of any type, or one plexiform neurofibroma
3. Freckling in the axillary or inguinal regions
4. Optic glioma
5. Two or more Lisch nodules (iris hamartomas)
6. A distinctive osseous lesion such as sphenoid dysplasia or tibial pseudarthrosis
7. A first-degree relative (parent, sib, or offspring) with Nf-1 as defined by the above criteria

Table 1. The NIH diagnostic criteria for neurofibromatosis type 1

Neurofibromatosis type 1 is caused by heterozygous mutations (intragenic or microdeletion) in *NF1* tumor suppressor gene, located at 17q11.2. Its product, neurofibromin, has different biochemical interactions, including association to microtubules and participation in several signaling pathways, especially as a member of the GTPase-activating proteins. Its main physiological function is inactivation of energized *ras* oncogene. *NF1* mutation extinguish a gene function and leads to aberrant *ras* activity. *NF1* gene belongs to the family of tumor suppressor genes and neurofibromatosis type 1 is thought to be a hereditary malignancy syndrome, which is highly influenced by complex action of other genes, required in signal transmission processing. In human diseases predisposing to cancer, cells usually carry heterozygous germline, what means inherited, mutations in growth regulator genes that are essential for organized cell growth and differentiation. Affected individuals, such as Nf-1 patients, are at significant risk for development of benign or malignant tumors early in life. In case of Nf-1, the most distinguished types of neoplasia are tumors arising from peripheral and optic nerves sheath (Schwann cells), usually benign neurofibroma and optic nerve glioma or seldom, malignant peripheral nerve sheath tumor [MPNST]. MPNST usually growths as a result of malignant transformation of plexiform neurofibroma [PNF], a specific, clinically distinguished type of neurofibroma. The risk of transformation of PNF into MPNST is not higher than 10%, and have been finally evaluated in recent clinical trials (Upadhyaya, 2011). The overall risk of cancer development in Nf-1 patients surpass the healthy general population risk by 2.7 times (Walker et al., 2006). The risk of malignancy is higher in those patients because inherited nature of the first mutation released the entire process, and in consequence, only one additional acquire genetic alteration, resulted in loss of the wild allele of affected gene, is further necessary to facilitate tumorigenesis. In healthy

individual both mutations must be acquire in intact alleles, so the chance for that is much lower. Cells that have lost both copies of the tumor suppressor gene have a growth advantage over so called wild cells. In a susceptible environment, this 'second hit' may result in tumor formation (Larizza et al., 2009; Upadhyaya, 2011). This helps to explain the development of neurofibromas and other malignancies occurring in Nf-1 patients, but currently does not fully explain the role of neurofibromin on the development of other ailments, and osseous abnormalities in particular. However the precise role of neurofibromin is not yet fully elucidated, although neurofibromin deficiency causes multiple clinical effects, suggesting that this gene product has diverse functions in a variety of tissues. Distorted process is responsible not only for tumorigenesis, but also for memory processing (intellectual disability) and bone remodeling (typical bone deformity seen in Nf-1 patients) (Radtke et al., 2007).

Phenotype expression of *NF1* gene mutation is extremely variable, so even individuals from one family with identical germline mutation may have dramatically different clinical manifestations. Observed complexity and the diversity of constitutional *NF1* mutations occurring in Nf-1 patients will continue to make genotype-phenotype correlation almost impossible. Clinical variability of Nf-1 results most probably from a combination of founder mutation effect of *NF1* gene influenced by further action of other genes engaged in signal transduction, as well as other genetic, non-genetic, and stochastic factors (Jouhilahti et al., 2011). Till now more than 500 different mutations of the *NF1* gene have been identified, and most of them are unique to a particular family. Different, mostly loss-of-function mutations, have been observed repeatedly, but none has been found in more than a few percent of studied families (Radtke et al., 2007). In consequence, there are not so called hot spots sequences manifest along the entire gene length, what significantly complicated not only molecular testing but genetic counseling as well. Till now, only two clear correlations have been observed between particular mutant *NF1* alleles and consistent clinical phenotypes. In one, the whole *NF1* gene deletion is associated with more prominent presentation of the disease (Mensink et al., 2006). In another, characterized by 3-bp in-frame deletion of exon 17 (c.2970-2972 delAAT), the typical pigmentary features, but no cutaneous or surface plexiform neurofibromas, exist (Upadhyaya et al., 2007). Data concerning *NF1* mutation have accumulated slowly owing to the variability of the mutation types and the size and complexity of the gene, belongs to the longest in human genome. This also reflects the lack of a simple, inexpensive, highly accurate DNA-based test for Nf-1 at present (Radtke et al., 2007).

Currently no treatments dedicated specifically to Nf-1 patients exist. Because of the increased risk of cancer and multiorgan involvement, Nf-1 patients required extensive medical surveillance, provided on the regular bases by the specific, highly specialized Nf-1 clinics (Karwacki & Wozniak, 2006). It obey a comprehensive standards, comprises of regular physical examination by a Nf-1 specialist, regular blood pressure and ophthalmologic monitoring, anthropometric and developmental assessment of children and periodical imaging, warrants follow-up of clinically suspected intracranial and other internal tumors, done by USG and/or MR imaging. CT scans are hardly recommended in NF-1 patients, but children in particular, as the imaging exerts the risk of irradiation and is of limited diagnostic value, especially in visualization of Nf-1 brain specific lesions, called undifferentiated bright objects [UBO](DeBella et al., 2000a). According to specific ailment, patients are referred by Nf-1 clinic to other specialists as well.

The skeletal manifestations of a disease itself and the post-surgical bony complications occurred in Nf-1 patients, are common and have a prominent place in the orthopedic literature. The orthopedic complications of Nf-1, which usually appear early, include spinal deformities such as dystrophic scoliosis and kyphoscoliosis, congenital bowing and pseudoarthrosis of the tibia and the forearm, overgrowth phenomenon of the extremity, and soft tissue tumors, both benign and malignant (Crawford & Schorry, 1999, 2006).

2. Primary and secondary bone abnormalities in neurofibromatosis type 1

Although phenotype of Nf-1 patients is well described, the osseous manifestations are rarely emphasized in the clinical and genetic discussions concerning the diseases. In fact, one of the seventh diagnostic NIH criterion represents a distinctive osseous defect. Nf-1 is classically considers as a neurocutaneous disorder and clinical features of Nf-1 are classically thought of as neural crest in origin, but currently it is well accepted that mesodermally-derived abnormalities coexist as well. Restricted knowledge concerning the pathophysiology of Nf-1 skeletal abnormalities reflects the limited therapeutic modalities. Nf-1 is characterized by a multifaceted, polysystemic pathology. Among the other, bone involvement is representative for the majority of patients with Nf-1. The presentation of an ailment differs from patient to patient, but sometimes is extremely severe and appear from the birth or become evident either early in the childhood or further on and will accentuate with age. A number of skeletal involvements are highly morbid with a natural history distinct from that of the general population. A large proportion of patients with Nf-1 display primary skeletal involvement, including scoliosis and pseudoarthrosis, which are compounded by osteoporosis and poor bone healing (Crawford & Schorry, 1999). In considerable proportion of patients, these bone lesions can result in significant morbidity. The natural history and pathogenesis of the skeletal abnormalities, resulted from alter *NF1* gene function, are poorly understood. Consequently, therapeutic options for these ailments are currently limited. Corrective orthopedic intervention quite often fails necessitating multiple revision surgeries followed by prolonged recovery periods (Crawford & Schorry, 2006).

Besides true dysplasia of bone, some of the skeletal changes observed in these patients are secondary to a tumor, compressing the bone through expansive growth, or its metastases. The most frequently, such tumors are plexiform neurofibromas, and rarely other malignancies occurred in Nf-1 more frequently than in general population, such as soft tissue sarcomas, notably rhabdomyosarcoma, and especially, malignant peripheral nerve sheath tumor [MPNST]. These tumors infiltrate easily in surrounding tissue, eroding the neighborhood bone, and frequently give rise to metastases, mostly to bones. Malignant peripheral nerve sheath tumor is an uncommon soft-tissue sarcoma [STS] that occurs at a higher incidence in patients with prior radiation exposure and Nf-1. MPNST resulted almost exclusively from the malignant transformation of PMF. It is assessed that every Nf-1 patient presenting PMF has a life time risk of 8 to 13% to develop a MPNST out of a pre-existing benign plexiform neurofibroma. In comparison to intragenic mutation, in patients with a *NF1* microdeletion (5% of Nf-1 patients) this risk is twice as high. That risk is even greater in patients, in whom PMFs were incorporated into the field of therapeutic radiotherapy performed as an component of complex oncological treatment. Irradiation is linked with much higher risk of such a transformation, and in some STS therapeutic protocols introduced to the clinic by oncological treatment groups is either contraindicated or introduced with caution, when offered to children with Nf-1. Most of MPNST, especially

arising in Nf-1 patients, are considered high-grade sarcomas with the tendency to recur as well as to metastasize, typically to the lungs. It belongs to a group of malignancies of particularly worse prognosis and due to its rarity, there is a paucity of data concerning chemotherapy response of MPNST.

As the overall understanding of bone growth, remodeling, and repair dependent on *NF1* gene function is critical to development of possible therapeutic interventions, in order to ensure continued collaboration and advancement toward better clinical management as well as effective drug therapies for Nf-1 related primary skeletal ailments, an International Nf-1 Bone Abnormalities Consortium have been convened in February 2008. The main goal of this Consortium is to identify barriers that might be impeding progression to future clinical trials for Nf-1 skeletal abnormalities and to highlights priorities for future research, based on animal model of Nf-1 related bone disease (Elefteriou et al., 2009).

2.1 Skeletal abnormalities in humans: Clinical presentation, diagnosis, complications

The Nf-1 skeletal phenotypes might be either generalized or focal. Manifestation of generalized skeletal abnormalities, mostly osteopenia or osteoporosis and short stature, are common, but of mild clinical implications. Focal lesions, such as tibial dysplasia, short angle scoliosis, and sphenoid wing dysplasia, are less common, but usually cause significant morbidity.

Skeletal Deformities:
- Short stature
- Macrocephaly
- Sphenoid wing dysplasia
- Cervical spine disorders
- Scoliosis
- short-angle dystrophic
- idiopathic non-dystrophic
- Spondylolisthesis
- Kyphoscoliosis
- Abnormalities of the rib cage and/or rib fusion
- Long bone dysplasia
- Osteosclerosis
- Congenital bone bowing
- Pseudoarthrosis
- Genu varum/valgum
- Absence of the patella
- Subperiosteal bone proliferation
- Partial overgrowth of an extremity
- Syndactyly
- Intra osseous-cysts and lytic bone lesions
Bone Metabolism Disorders:
- Osteopenia and frank osteoporosis
- Hypophosphatemic rickets
- Impaired bone healing

Table 2. Frequent manifestations of osseous abnormalities in patients suffering from Nf-1

2.1.1 Focal lesions: Spinal and chest wall deformations

Spinal deformities frequently occur in individuals with Nf-1. These changes result from intra or perispinal pathology, such as tumors, or either meningoceles or dural ectasia. However, the deformities may be also present in persons with entirely normal intraspinal contents. In such patients, primary bone dysplasia accounts for the dystrophic vertebral changes.

The most frequent is scoliosis, and the most devastating form – kyphoscoliosis of progressive course regardless the intensive physiotherapy. In various series of Nf-1 patients reported in the literature, frequency of scoliosis is assumed for 10 to 33% (Crawford & Herrera-Soto, 2007; Wang & Liu, 2010). Vice versa, in general population, Nf-1 could be confirm in app. 2% of children suffering from scoliosis (Vitale et al., 2002). Orthopedic surgeons distinguished two types of spinal curvature disturbances in children with Nf-1: dystrophic and non-dystrophic. The cause of spinal deformity in Nf-1 is still a matter of debate, but some have suggested that it is secondary to endocrine disturbances observed in these patients, mesodermal dysplasia probably resulted from *NF1* mutations, and osteomalacia, caused by a localized neurofibromatous tumor eroding and infiltrating adjacent bone.

The dystrophic scoliosis, usually associated with paravertebral neurofibromas, has a progressive nature and is associated with vertebral scalloping and wedging. Almost always develops before 10th year of life (Crawford & Herrera-Soto, 2007). Dystrophic scoliosis is often early onsetting, the shortsegmented, sharply angulated type of this ailment that includes fewer than 6 spinal segments. It has a tendency to progress to a severe deformity. The term dystrophic is usually used to describe a dysplastic vertebrae observed within scoliotic spine. Although there is no formal diagnostic criterion for such a form of scoliosis, Durrani et al. (2000) described nine specific radiographic features associated with dystrophic scoliosis (Tabl. 3).

1.	Scalloping of the posterior vertebral margins
2.	Severe rotation of the apical vertebra
3.	Widening of the spinal canal
4.	Enlargement of the neural foramina
5.	Defective pedicles
6.	A paraspinal mass
7.	Spindling of the transverse process
8.	Rotation of the ribs (the ribs resemble twisted ribbons)

Table 3. The radiologic appearance of the dystrophic scoliosis (Durrani et al., 2000)

Distinctive radiographic features of dystrophic scoliosis, usually presented in the preadolescent child, include a short-segment sharply angulated curve (involving four to six vertebrae), scalloping of vertebral margins, vertebral wedging, spinal canal widening, defective pedicles, and rib-penciling (Crawford et al., 2007). It is potentially debilitating and may rapidly progress to neurological impairment. This kind of scoliosis is frequently associated with paraspinal or other internal neurofibromas adjacent to the vertebrae, which could be seen in app. 70% of Nf-1 patients' MRI (Khong et al., 2003; Ramachandran et al., 2004). The complication of NFMs, or much frequently, PNFs penetrating into vertebral canal is dural ectasia, defined as widening of the dural sac surrounding the spinal cord, which might be seen in these patients (Khong et al., 2003; Schonauer et al., 2000; Tubbs and Oakes,

2002). However, dural ectasia may be a primary mesodermal dysplasia of the meninges as well (Casselman and Mandell, 1979). The vertebral column can further displace or erode, causing rib dislocation into the spinal canal, resulting in spinal cord injury. Weakening of spinal natural stabilizers, such as facets, pedicles, and ligaments, usually distorted in Nf-1, may lead to kyphosis. Kyphoscoliosis and humpback is a severe complication of advanced dystrophic scoliosis and finally can lead to cardiorespiratory insufficiency and failure. In this point, the best and well known touching description of Nf-1 patient's suffering, given by Victor Hugo in "The Hunchback of Notre Dame", is worth to be remembered. Dystrophic scoliosis in Nf-1 patients is particularly difficult to treat and necessitates early aggressive surgical stabilization very often.

The other, milder form of scoliosis occurring in Nf-1 children, is called non-dystrophic. It is diagnosed typically during adolescence and resembles idiopathic adolescent scoliosis in healthy population (Crawford & Herrera-Soto, 2007; Wang & Liu, 2010). This form usually involves 8-10 spinal segments. The deformity is most often convex to the right; however, this is not consistent. In rare instances, non-dystrophic scoliosis can progress to the dystrophic form.

The presence of neurofibroma or abnormal pressure phenomena in and around the spinal canal neuraxis resulted in meningoceles, pseudomeningoceles, dural ectasia, and dumbbell lesions development.

Kyphosis in individuals with Nf-1 might be distinguished by acute anteroposterior angulation. Severe deformity of vertebral bodies in Nf-1 might be confused even with congenital deformities.

Chest wall deformities in patients with Nf-1 are observed even more frequently than scoliosis, and are thought to be present in as many as 50% of patients (Riccardi, 2010). The relationship between chest wall deformities and scoliosis is not clear, but its existence exacerbates the course of dysplastic scoliosis in particular. It could happened, that chest wall deformities constitute the first clinical sign of tumor arising within the chest and quite often penetrating throughout the intervertebral foramina or dura mater. It resembles an hourglass shape, and is called spinal dumbbell tumor. This kind of growth is usually form by multiple tumors arose in the intradural and epidural spaces from one nerve root, occurring at the same time in different regions, such as the paravertebral, epidural and intradural spaces. Histopathological diagnosis is usually plexiform neurofibroma, but it still comprises the risk of malignant transformation into MPNST.

2.1.2 Focal lesions: Head and neck region

Increased head circumference is frequently observed in patients with Nf-1, and macrocephaly (head circumference >2 SD above the mean) occurs in about one-fourth of patients (Szudek et al., 2000). It is thought to be the consequence of brain enlargement (Greenwood et al., 2005). It is still not clear whether the skull growth contributes to macrocephaly. Contrariously, the association between macrocephaly and learning disabilities or underlying structural brain abnormalities has never been proof (Gutmann at al., 1997).

Sphenoid Wing Dysplasia Cranial defects attributed to the clinical pathology in Nf-1 with relatively lower frequency (eg. 11% had a dysplastic sphenoid wing in an observational study of Friedman and Birch (1997)). Sphenoid dysplasia usually is asymptomatic but occasionally can be associated with herniation through the bony defect. It is still under debate whether these type of changes reveal a primary bone dysplasia related to *NF1*

mutation, or occur as secondary response of bone to the adjacent soft tissue abnormality. Most cranial defects are associated with plexiform neurofibromas of the eyelid or temporal region induced ipsilateral infiltration and decalcification of cranial bones adjacent to tumors (Jacquemin et al., 2002, 2003). Other lesions, including arachnoid cysts, dural ectasia, or buphthalmos, usually associate sphenoid wing defects. The suggestion of a bone cell-autonomous defect, accounted for the dysplastic sphenoid wing, is based on two meaningful observations: (1) Nf-1 sphenoid wing lesions have been associated with tibial and vertebral dysplasia (Alwan et al., 2007), and (2) formation of this skull structure proceeds through endochondral bone formation, which is defective in Nf-1 (Kolanczyk et al., 2007). Regardless the cause, a congenital malformation or secondary bony defect, the sphenoid wing dysplasia is not currently a primary target for therapeutic prevention. Nevertheless, it is imperious necessity to apply sensitive imaging techniques to screen patients with sphenoid wing dysplasia for adjacent tumors, which may be amenable to therapy (Jacquemin, 2002, 2003).

Increased caries and early primary tooth eruption as well as periapical cemental dysplasia have been quite often reported in patients with Nf-1 (Lammert et al., 2007; Tucker et al., 2007; Visnapuu et al., 2007). Dental abnormalities in Nf-1 patients still require more attention, yet everyday practice pointed unnecessary dental procedures performed in these patients, for instance when periapical cemental dysplasia is confused with chronic inflammation on radiographic analysis and precipitate dental surgery.

Cervical spine abnormalities are due to cervical spine instability or intraspinal pathology, caused mostly by benign tumor. These occur much more frequently when a scoliosis or kyphoscoliosis is present in the thoracolumbar region, but could be omitted as the examiner's attention is focused on the more obvious deformity. Severe cervical kyphosis is the most common abnormality, which itself is highly suggestive for Nf-1 diagnosis. Patients usually had either limited motion or pain in the neck, which were probably attributed to cervical instability. The numerous, minor to major neurologic deficits, such as paraplegia, are present.

2.1.3 Focal lesions: Long bones and extremities

Long bone dysplasia appears in a small percentage (3–4%) of patients with Nf-1 in clinic-based series (Friedman & Birch, 1997) and tibia is involved most often among other long bones, which can be affected sparsely. Infant with such an ailment usually presents with unilateral anterolateral bowing of the lower leg, notably tibial, although a child may be born with fracture and/or pseudarthrosis as well, or develop these shortly after birth. The deformity may appear before other protean manifestations of Nf-1, such as café-au-lait spots. The tibial bowing is usually evident within the first year of life, with a fracture not uncommonly occurring by age of 2-2.5 year. The tibial bowing associated with Nf-1 is always anterolateral. Affected bone is subject to pathologic fracture usually before age 3 years, often with minimal trauma. Subsequent healing may not occur normally, leading to consecutive non-union and pseudoarthrosis, sometimes requiring even amputation of affected extremity. As confirm histologically, the fibrous pseudarthrosis tissue seen at the fracture site is not a neurofibroma, but a fibrous overgrowth of unspecified cell origin. The ipsilateral fibula is often involved in association with tibial pseudoarthrosis and focal dysplasia of the ulna, radius, scapula, or vertebra may occur as well. The anterolateral bowing characterized patients with Nf-1 should be distinguished from the bilateral

physiological bowing, exemplary common in children as they begin to walk or from the other type pathology.
Various radiographic classification systems for tibia bowing have been proposed, but they are still not rigorous and several subtypes represent rather changes over time, than the real variety. Nevertheless, tibial bowing in Nf-1 patients prior to fracture represents in radiograph a cortical thickening and medullary canal narrowing at the apex of the convexity, typically near the junction of the middle and distal thirds of the tibia (Stevenson et al., 2007).
In general, every bone of whatever kind and at any localization may usually be affected by the adjacent tumors as well, with all the consequences resemble the ones described above.

2.1.4 Generalized skeletal abnormalities

Although focal skeletal abnormalities, such as dystrophic scoliosis or tibial pseudoarthrosis and the like, can be severely disabling, they are uncommon among individuals with Nf-1. In contrary, generalized skeletal abnormalities are less severe but much more frequent in these patients. The osseous dysplasia result from disturbed bone growth, perhaps secondary to a mineralization disturbance. Findings such as decreased bone mineral density (BMD) and short stature reflect a generalized alteration of bone. The Nf-1 patients tend to be below average in height for age (below -2SD), although heights less than -3SD below the mean is seen hardly ever. Decreased BMD in both sexes at an early age has been reported in up to 50% of individuals with Nf-1. Reduced BMD in Nf-1 patients was initially recognized by Illes et al. (2001).
The exact pathogenesis of these bony changes is not understood, but patients with Nf-1 present lower than expected serum 25-hydroxyvitamin D (25OHD) concentrations, elevated serum parathyroid hormone concentration, and evidence of increased bone resorption. Defects in vitamin D metabolism, osteoclastogenesis or bone cell response to systemic signals regulating bone remodeling are likely involved. An inadequate increase in bone remodeling is also indirectly confirm by both bone histomorphometry and changes in circulating bone markers (Stevenson et al., 2008; Seitz et al., 2010). However, an increased incidence of fractures has not been firmly established. Still, generalized osteopenia and frank osteoporosis are more common than expected in patients with Nf-1. The results of one of the biggest series, in which Nf-1 children became the subject of multivariant analysis, indicate that the mean lumbar and whole body BMD z-scores were in the range of osteopenia and osteoporosis in 48% and 25% of subjects, respectively. BMD was reduced at multiple bone sites, while the lumbar spine being more severely affected (Brunetti-Pierri et al., 2008). A tumor inductive role has also been suggested (Ben-Baruch et al., 1994).
Several case reports from Nf-1 patients have identified histologically proven osteomalacia, which might be associated with hypophosphatemia due to renal phosphate wasting (Abdel-Wanis & Kawahara, 2002). Although the concentration of baseline vitamin D were in the normal range in these patients, researchers further found that the osteomalacia can be reversed independently of phosphate supplementation with oral treatment of 1-alpha-(OH)-vitamin D3 (Konishi et al., 1991). Moreover, a recently published comparative study reported that 25OHD serum concentration were about twofold lower in the Nf-1 patients than in healthy population and were inversely correlated with the number of neurofibromas (Lammert et al., 2006). The exact underlying mechanism of vitamin D deficiency in Nf-1 patients still remains unclear as well as the value of vitamin D supplementation for the treatment of the patients is still poorly understood.

Selected genetic disease populations, including Nf-1, display increased risks for osteoporosis, which is an emerging complication of utmost importance. Early diagnosis in the pediatric population is essential, since the highest contribution to peak bone mass is attained in the first three decades. Osteopenia or decreased bone mass accrual in the pediatric period can lead to frank osteoporosis and fractures in adulthood in the general population, as peak bone density is generally reached by late adolescence. Emerging evidence shows that vitamin D deficiency combined with a higher than normal bone turnover contributes to decreased bone mineral density in patients with Nf-1. The results of currently published studies suggest that the population of Nf-1 patient is at an increased risk for the development of clinical complications related to osteoporosis.

Seldom but yet published data revealed that significantly lower blood concentration of osteocalcin was observed in Nf-1 patients with, in comparison with patients without, skeletal abnormalities. Osteocalcin is secreted by osteoblasts, plays a role in mineralization and calcium ion homeostasis and its level reflects the rate of bone formation. Reduced blood concentration of osteocalcin in Nf-1 patients with skeletal deformity may indicate defect in osteoblasts functioning. Other biochemical markers of bone turnover usually do not exhibit any difference between these groups (Duman et al., 2008). When compared to healthy subjects, in Nf-1 patients BMD of the lumbar spine and femoral neck is significantly decreased. The same significant decrease apply to pubertal patients when compared to pubertal controls and in prepubertal patients when compared to prepubertal controls. The decrease in BMD is still more pronounced in Nf-1 patients with severe scoliosis, than those without spinal deformities. Duman et al. (2008) suggests that relevant predictors of skeletal abnormalities among Nf-1 patients are bone formation markers (exclusively osteocalcin) rather than imaging (conventional radiography, CT, MRI or quantitative ultrasonometry of the calcaneal bone) and densitometry techniques, especially dual-energy X-ray absorptiometry (DXA). In opposite to this report, the other published data suggest that both DXA and quantitative ultrasonometry of the calcaneal or other bones may prove useful to identify individuals with NF1 who are at risk for clinical osseous complications. These techniques and the logistics, introduced into pediatric practice quite recently, may also be appropriate for monitoring of therapeutic trials concerning skeletal ailments in Nf-1 children. However, many significant heterogeneities among the reports in the literature, such as patient groups (ages, variability of skeletal involvements, etc.) and methods (BMD assaying, comparison criteria such as T-score, Z-score, their cutoff points, etc.) make the comparisons amongst the studies very difficult. The densitometric criterion commonly used as a predictor of fracture risk for osteoporotic adults, called T-scores, derived from reference populations of young-adult women. While useful for evaluation of fracture risk in adults, but especially in postmenopausal women, is not applicable to the diagnosis of osteoporosis in children. In this age period the evaluation of osteoporotic risk of fracture is much more difficult. Densitometric data in children must be compared with age-matched control populations (z-scores). Currently, it is generally accepted that z-scores below −1.5 indicate low bone mass or osteopenia, and that osteoporosis is suspected strongly with z-scores below −2, especially followed by the episodes of fracturing. According to Writing Group for the ISCD Position Development Conference, z-score less than −2 define low bone density in children (2004).

According to unique published papers, children with Nf-1 had also statistically significant decreases in muscle mass compared to healthy controls regardless the presence or not of

clinically proven osseous abnormalities (Stevenson et al., 2005). Muscle mass is important in the development of bone strength, as voluntary muscle forces (the largest physiological load) impact skeletal response. Combinations of extrinsic forces including decreased muscle mass could compromise potentially abnormal osseous matrix as well.
It is well known that *NF1* gene is widely expressed in chondrocytes, osteoblasts, osteoclast, and osteocytes (Kuorilehto et al., 2004). Kuorilehto et al. (2004) reported as well that neurofibromin is expressed in growing cartilage in areas where proliferation has ceased and the chondrocytes are undergoing differentiation, and in periostealosteoblasts of embryonic and mature mice and rats. However, mechanism of various skeletal deformities and bone metabolism defects in Nf-1 patients are not clearly understood. Experimental work done on animal models suggest that patients with Nf-1 suffers from a bone formation defect rather than bone resorption.

2.1.5 Other skeletal manifestations of neurofibromatosis type 1

Individuals with Nf-1 tend to be shorter than expected for their families (Szudek et al., 2000; Virdis et al., 2003), with 20–30% of adults with NF1 estimated to have a height below the 3rd centile. Growth velocity in these individuals is typically normal or near normal before puberty, then declines. Short stature in patients with NF1 is usually proportional. Scoliosis, growth hormone deficiency, and other Nf-1 related complications can contribute to short stature, but the cause of this in most patients with Nf-1 is unknown.
Bony abnormalities may be clinically silent, with radiographic evidence of long bone intramedullary fibrosis, cortical thinning, or vertebral dural ectasias often found incidentally.
Among the other bone abnormalities observed Nf-1 patients rarely, but with frequency a bit higher than in general populations, are cystic osseous lesions. They are usually identified incidentally during ongoing process of repeated imaging, reflecting the international recommendations. Found during radiographic knee exam, these cystic lesions are occasionally seen in the absence of tumors or long bone dysplasia (Colby & Saul, 2003; Lee & Cho, 2006). The lesions rarely fracture or show progressive deformity, and biopsy generally shows non-ossifying fibroma bone tumors. The association of multiple non-ossifying fibromas with cafe au lait skin patches are the fundamental signs of Jaffee-Campanacci syndrome (JCS) (Campanacci et al., 1983). The long bones affected more often are the femur, the humerus, and the tibia as well as the bones of the jaw. Other bones can be involved less frequently, especially the pelvis, the fibula, the radius, and the ulna. The lesions may be large enough to cause pathological fracture of the involved bone. Recent findings suggest that JCS may be a form of Nf-1 (Colby & Saul, 2003).

2.2 Pathophysiology of skeletal abnormalities in neurofibromatosis type 1: Experience from transgenic mouse models

Studies assessing the role of *NF1* gene not only in tumor formation and development, but also in pathogenesis of other multiple abnormalities, are to be a matter of numerous experimental work, which cannot be apply neither on living individuals, nor cell lines. These restrictions led to the development of transgenic mouse models allowing determination of the role of *NF1* gene and its product in affected systems and facilitate preclinical studies. Unfortunately, mice's embryos with inactivated both *NF1* alleles exhibit severe neural closure defect, namely exencephaly, and cardiovascular abnormalities including structural malformations of the outflow tract of the heart and enlarged

endocardial cushions. These *NF1*-deficient embryos die between embryonic days 12.5 and 13.5, presumably due to the cardiac vessel defect. In contrast to completely defective organism, inactivation of only one allele of *NF1* gene locally, in the neural crest only, does not cause cardiac defects but results in tumors of neural crest origin, resembling those seen in Nf-1 patients. Following this early experiments the another models of an experimental animal has been developed to determine the role of *NF1* in bone cells and resolve difficulties in understanding the human pathophysiology of Nf-1 skeletal defects (Kolanczyk, 2007, 2008).

It has been well known that neurofibromin is a cytoplasmic protein that is predominantly expressed in neurons, Schwann cells, oligodendrocytes, astrocytes, and leukocytes. Due to early studies based on transgenic animal models of skeletal defective mice it is obvious currently that *NF1*-mRNA and neurofibromin are expressed in mouse bone and cartilage during development and adulthood (Kuorilehto et al., 2004), and more specifically in mesenchymal stem cells, chondrocytes, osteoblasts (Elefteriou et al., 2006; Kolanczyk et al., 2007), and osteoclasts (Yang et al., 2006a). Kuorilehto et al. (2004) reported the expression of neurofibromin in growth plate, periosteum, and tracebular bone of mice, and the expression in growth plate was mainly located in chondrocytes of the hypertrophic layer. Yu et al (2005) showed that upon the activation of *ras* signals, *NF1*+/- murine osteoprogenitor cells show increased proliferation and premature apoptosis; the osteoprogenitor cells also exhibit a lower rate of differentiation to osteoblasts. He considered neurofibromin and its role as *ras* signal regulator to be necessary for osteoblast function. Kolanczyk et al (2007) found that osteoblasts from *NF1*Prx1(*NF1*+/-) mice show increased proliferation and decreased abilities to differentiate and mineralize, whereas chondrocytes demonstrate a lower proliferation rate and defective differentiation. These results indicate that *NF1* has multiple roles in skeletal development including joint formation, growth plate function, osteoblast differentiation, and control of vessel growth, and proved that *NF1* is an important regulator of development and growth of the skeleton. The pattern of expression suggests that *NF1*-related skeletal abnormalities stem in part from primary osseous defects caused by bone cellular dysfunctions related to generalized *NF1* heterozygosity, and/or to *NF1* loss of function in specific bone cell types.

Investigations of affected skeletal tissue in tibial pseudarthrosis model created by inactivation of one of *NF1* alleles during early mouse limb development, confirm that further mutation of the second *NF1* allele, thus the homozygous loss of *NF1* function, was detrimental for normal bone development. Thus, like in *NF1*-mediated tumorigenicity, a loss of both *NF1* alleles is likely to be required to cause the skeletal abnormality phenotype.

Available mouse models recapitulate some, but not all, of the bone abnormalities in patients with Nf-1. Mouse data helped clarify that *NF1* haploinsufficiency is likely related to the generalized Nf-1 bone remodeling defects, whereas total loss of *NF1* function is likely related to the focal dysplastic events. Identifying neurofibromin cellular functions, target genes and downstream signaling pathways remains a priority to understand the etiology of the Nf-1 skeletal manifestations.

3. Present day and future treatment of skeletal dysplasia in neurofibromatosis type 1

Consensus guidelines for the treatment of the specific orthopedic manifestations in patients with Nf-1 do not exist and clinical management practices for each Nf-1 skeletal abnormality varied considerably.

At present, there are either no clinical trials to support the use of osteoporotic drugs in a population of Nf-1 patients or treatment guidelines. Thus, conservative therapy to promote bone health, such as treatment with calcium and vitamin D, and weight-bearing exercise forms the first line of therapy in children with Nf-1 and low bone mass. Correction of measured deficiencies in hormones (vitamin D, thyroid, estrogen, etc.) that are known to regulate skeletal growth and maturation is inevitable. Judicious vitamin D supplementation may prove beneficial for patients with Nf-1 who have vitamin D deficiency or evidence of osteopenia. Similarly, until further information is obtained, treatment of osteoporosis in adults with Nf-1 follow the recommendations developed for general population. Because low serum 25-OH-D and osteomalacia has been reported in patients with Nf-1 and low bone mass, osteoporotic adult patients over age 50 years should take supplemental 1,200 mg calcium and 800–1,000 IU vitamin D per day, and reduce clinical risk factors by regular weight bearing and muscle strengthening exercises and avoidance of smoking and excessive alcohol. Selection of approved anabolic or anti-resorptive drugs to prevent or treat osteoporotic fractures should follow the standard practice with exception of children. Children should be treated as well, as soon as fracture complicates the osteopenic/osteoporotic bone dysplasia (Elefteriou, 2009). Unfortunately, anabolic substances available currently upon two forms of parathyroid hormone pose the increased risk of osteosarcoma development, proven in rats, and are contraindicated in children (Tashjian & Gagel, 2006). Bisphosphonates and monoclonal antibodies that target osteoclasts, belonging to anti-resorptive drugs, have been used to reduce osteoporotic fractures in adults, but their effect on BMD and fracture risk in children with Nf-1 is unknown. Clinical trials are necessary to determine which of currently available therapies are most effective to treat patients with Nf-1 and reduces BMD, but especially frank osteoporosis.

In patients with Nf-1, the morbidity associated with either dystrophic scoliosis or tibial dysplasia is much greater than that of osteopenia and osteoporosis or even non-dystrophic scoliosis. This helps define priorities for current and future trials. Some of the ailments, particularly dystrophic scoliosis or tibial bowing, can lead to clinically significant consequences if neglected.

Children with dystrophic scoliosis require the extensive medical attention. In children who do not complete skeletal maturation, typically presented dystrophic form of scoliosis, bracing is routinely used when the spine curvature exceeded 25 up to 45 degrees. While the curvature progresses to more than 45 degrees before maturity or 55 degrees after maturity, surgery is commonly employed. Unique management approach due to progression is necessary in skeletally immature patients with dystrophic scoliosis in whom a curvature exciding 30 degrees. Exclusion of paravertebral tumors and dystrophic changes required MR or, less sensitive, CT imagings, as those finding may be missed on plain radiographs. Regarding possible complications of surgery, careful presurgical assessment is critical, as the lamina may be thin, the canal affected by dural ectasia or intraspinal tumors, and a rib may have displaced into the spinal canal, all of which express an increased risk of poor post-surgical outcome. Variables such as age, gender, associated neurofibromas, location and degree of the curve, and associated radiographic dystrophic features make the operation design difficult. Surgical treatment with fusion and growing rods is complex. Occasionally, intraspinal elements may directly compromise the cord when instrumentation and stabilization are attempted, or they may cause erosive changes in the bone, preventing

primary fusion. The local condition may exclude the possibility of radical excision of tumor not infrequently, additionally worsen the postsurgical outcome. A lack of animal models of dystrophic scoliosis and consequently poor understanding of the natural history of this ailment, additionally hinder progress. As the pathogenesis of Nf-1 dystrophic scoliosis is still poorly understood, there are no clear pharmacologic adjunctive options. It is postulated, that prospective studies to determine the relationship of spinal neurofibromas in patients with dystrophic scoliosis may help to determine if early treatment of spinal tumors could prevent dystrophic scoliosis. Currently there is no effective treatment for Nf-1 related dural ectasia. If microfractures and vertebral wedging with subsequent development of scoliosis is diagnosed, then pharmacologic agents to increase vertebral strength may be appropriate (Elefteriou, 2009).

The dumbbell tumors, most of which are located unilaterally in the spinal canal and paravertebral space, are excised through a hemilaminectomy and a facetectomy, because these techniques provide large space for tumors excision. In addition, the spinal stability can be reconstructed by Rogers wiring and contralateral facet fusion, because the hemilaminectomy and facetectomy can minimize damage to spinal stability by leaving the spinous process, supra- and intraspinous ligaments, and contralateral facet joint.

Some dermal and most often internal plexiform neurofibromas, generally larger, more diffuse, and locally invasive to adjacent tissue and bone are seen in more than one fourth of patients with Nf-1 and can present a surgical or medical management conundrum. Besides pain, disfigurement, neurological and other clinical deficits complicated its growth, the wisdom of watchful waiting versus aggressive intervention is often debated (Wozniak & Karwacki, 2008). Complete resection of a PNF, radicalism of which is always controversial, without residual functional deficits is rarely possible, on the other hand, it must be remembered that app. 10% of them undergo malignant transformation. Thus debulking or partial resection of PNF may be undertaken not only for cosmetic purposes, but especially when progressive functional consequences are anticipated.

Surgical treatment of the chest wall deformities is usually not required, and the ailment, as well as short but proportional stature and not prominent macrocephaly, are assumed as principally cosmetic.

Sphenoid wing dysplasia, comprising a congenital malformation or a secondary bony defect, is not a primary target for therapeutic prevention. Although, it requires sensitive imaging techniques, particularly MRI, to screen patient for adjacent tumor, which may be amenable to therapy.

The management of anterolateral bowing deformity, characteristic for Nf-1, is most frustrating. Unlike scoliosis, treatment of congenital pseudarthrosis of the tibia does not appear to be more successful when it is initiated early. Anecdotally, early surgical intervention in children with Nf-1 and tibial pseudarthrosis results in poorer outcomes compared to later surgical management. The Consortium orthopedists recommended routine bracing of the dysplastic long bone upon diagnosis of bowing and agreed that prophylactic surgery should be avoided (Elefteriou, 2009). So, the current standard for treatment of long bone bowing in children is bracing to prevent fracture. The majority of members of the Consortium advocated early bracing until the child achieves maturity and, in some cases, continued even into adulthood. Evaluations of brace type, duration of use, or long-term benefits have not been obvious. Treatment of long bone pseudarthrosis is often unsatisfactory and very often require multiple surgeries or ultimate amputation. It is general

belief that bracing after pathological fracture should continue, delaying surgery until mid-childhood, in fifth - eight year of age at earliest. Among the surgical procedures the most often applied are resection of the pseudarthrotic region and bone bridging with fixation via intramedullary stabilization devices, or free vascularized fibular grafting (contralateral or ipsilateral), or external fixation (e.g., Ilizarov technique), either alone or in combination with transankle fixation. Residual angular deformity, ankle stiffness, limb length discrepancy, refracture, and chronic pain are amongst the most severe complications of long bone pseudarthrosis. Attempts must been made to promote bone healing, always impaired in children with Nf-1 affected bones. Thus, electrical stimulation, varying periods of postoperative immobilization, supplemental bone grafting, and more sophisticated techniques, such as application of bone morphogenetic proteins and monocytic progenitors stem cells are under the routine or experimental options. Summarizing, tibial dysplasia with pseudarthrosis is still challenging Nf-1 skeletal manifestation required further extensive elaboration, on both scientific and everyday practice fields (Elefteriou, 2009).

Established transgenic mouse models of *NF1* gene and its protein dysfunctions opens up new vistas for a better understanding of the natural history and the development of new therapies and long-term orthopedic management essential to improve patient care. Based on data from these models, a variety of cell types and signaling pathways are likely to be involved in Nf-1 patients with bone manifestations. Therefore, combination therapies, using both anabolic and anti-catabolic medications, will likely give optimal results. For example, use of locally applied biological mediators (e.g., bone morphogenetic protein) at the time of surgery in patients with pseudarthrosis is an attractive option in order to avoid complications of systemic administration of pharmacologic agents. Unfortunately, no mouse model, even closely resembles the human skeletal manifestations, is fully identical, despite similarities with the human condition, in part due to the limitations of the genetic manipulations. Nevertheless, *NF1*-deficient mice are currently the only and highly valuable project in preclinical testing of candidate therapies for Nf-1 skeletal defects.

Various studies have been initiated until now in preclinical mouse models to assess the potential efficacy of selected drugs on bone formation, repair and remodeling. Even when they represent just an initial approach, the most promising demonstrated potential of bisphosphonates (such as zolendronic acid) and recombinant human bone morphogenetic proteins (rhBMPs), which induces bone and cartilage formation, for improved net bone production in an in vivo model of heterotopic bone formation (Schindeler et al., 2008, Schindeler et al., 2011). Bisphosphonates are currently approved for other applications, so they could transition rapidly to Nf-1 clinical trials. Kolanczyk et al. (2008) quite recently published data concerning lovastatin, which improves cortical bone injury healing defects observed in the *NF1*-deficient mice. The inhibition of Ras/Erk signaling by lovastatin and other statins in mouse model counteracts the Ras/Erk constitutive activation occurred in *NF1*-deficient osteoblasts (as in Schwann cells), and improves bone healing defects. His work established the base for future experiments aimed at the treatment of the focal Nf-1 bone changes with local statin's delivery (Weixi et al., 2010).

4. Final remarks and conclusions for the future

Although neurofibromatosis type 1 is associated with marked clinical variability, most affected children do well from the standpoint of their growth and development. Some features of Nf-1 are present at birth, and others are age related abnormalities of tissue

proliferation, which necessitate periodic monitoring to address ongoing health and developmental needs and to minimize the risk of serious medical complications. Among the most important and often debilitating are skeletal abnormalities. The skeleton is frequently affected in individuals with Nf-1, and some of these bone manifestations can result in significant morbidity and even profound invalidism. The natural history and pathogenesis of these skeletal abnormalities are still poorly understood and consequently therapeutic options for these manifestations are currently limited. Lately established transgenic mouse models as well as continuously developing new and improved imaging techniques warrants further achievements either in basic science concerning the complications of *NF1* mutation or clinical availability of diagnostic tools. The ongoing investigational trials, both preclinical and clinical as well as observational, gather significant number of participants, strengthen patient's belief for future improved care and therapy potentially freed them from often burdensome complications of disease course.

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18

Studies of Osteoporosis in Cancer Patients in Slovakia – Experience from Single Institute

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1. Introduction

Osteoporosis is a metabolic skeletal disease characterized by low bone mineral density (BMD), damage of bone microstructure, bone fragility resulting in increase risk of bone fractures. Epidemiologic data are continuously showing rising number of newly diagnostic patients with osteoporosis. The expected number of bone fractures due to osteoporosis is to be 6. 26 million in 2050, growth from 1. 56 million in 1990 (Payer et al., 2007). The fractures are usually localized in lumbar spine (or other vertebra), hip and forearm (wrist), The most serious is the fracture of proximal femur (hip), beacuse approximately 20% of these pateints die within one year after the fracture and almost 80% become dependent on some kind of care (Cooper, 1997). The precise number of vertebral pathological fractures is difficult to assess, because many of these fractures are asymptomatic. Despite this they increase mortality by 23% (Cooper, 2007). The wrist fracture do not increase the mortality. The incidence is much more frequent in women than in men (4 : 1). The increased frequency of osteoporosis is partly due to increase in absolute number of new patients and partly due to continualy improving diagnostic procedures. The new generation of equipments and laboratory technics more precisely identify patients with bone loss. In the same time improvement in public information leads to increasing number of densitometric examinations.

Bone tissue is highly active metabolic organ. The bone tissue remodelation (formation of new bone tissue and its degradation) is active and continual process. Very important role in regulation of this process have hormones (estrogens and androgens). The mostly understood and resolved is postmenopausal osteoporosis and the most important risk groups and factors were identified (Rizzoli et al., 2005).

Cancer patients, especially those with „hormone dependent“ disease (breast cancer, prostate cancer) or those with treatment interfering in hormonal metabolism (breast cancer, prostate cancer, thyroid cancer, ovarian cancer, germ cell tumor and others) are in inceased risk of disease or therapy induced osteoporosis. There are increased numbers of information and references on this topic.

The most advanced are data on patients with breast cancer, particularly those with early breast cancer (EBC) on adjuvant aromatase inhibitors (AI) therapy.

Women with breast cancer, especially those receiving aromatase inhibitors are at higher risk for bone loss and fracture. Postmenopausal women may already have multiple risk factors

for fracture, and breast cancer therapies compound these risk (Hadji & Bundred, 2007). Fractures can have serious clinical consequences including need for major surgery, increased morbidity and mortality, increased cost of disease management, and reduced quality of the life for patients (Body, 2011).
Additional group of patients in risk are those with prostate cancer on hormonal therapy, thyroid cancer (TC) after total or nearly total thyroidectomy on whole-life substitution therapy by oral thyroxine (T_4) and patients with germ cell tumors (GCT) after surgery and radiotherapy and/or chemotherapy.
We have started to measure BMD in patients with breast cancer (BC), prostate cancer, thyroid cancer (TC) and germ cell tumors few years ago. Some of the results are nearly mature and ready to be publish (EBC, TC, GCT) others need more patients and time of follow-up (PC).

2. Breast cancer

2.1 Introduction

Postmenopausal breast cancer patients are in high risk of osteoporosis in many reasons – primary diagnosis of breast cancer, then side-effects of anticancer therapy, postmenopausal status. These factors mean not just elevated risk of bone loss, osteoporosis, but especially risk of patological fractures. Many of postmenopausal breast cancer patients, especially those with early stage, with aromatase inhibitors (AI) adjuvant therapy have very good prognosis. The elevated risk of osteoporosis can lead to patological fractures which may markedly worsen their quality of life (Coleman et al., 2008).
Antagonizing estrogen in hormone-dependent breast cancer is well-known method of reducing tumor growth. Five years of treatment with tamoxifen, an antiestrogen or selective estrogen-receptor modulator (SERM), has been shown to reduce the risk of recurrence and breast cancer mortality by 41% and 34% respectively and is still recommended as one of several options for early-stage hormone receptor-positive breast cancer.
New data from clinical trials comparing third-generation aromatase inhibitors (AI) with tamoxifen have confirmed that AI offer significant efficacy and tolerability advantage over tamoxifen. Aromatase inhibitors are recommended as adjuvant treatmen for postmenopausal women with hormone-receptor positive early breast cancer. The group of clinicaly used AI contains non-steroidal AI letrozole and anastrozole and steroidal-AI exemestane. The primary mechanism of action of AI is inhibition of aromatase activity. Aromatase is the most important enzyme responsible for conversion of androgens to estrogens, mainly in tissues outside endocrine system. This is the most important mechanism of estrogen production in postmenopausal women. Estrogen production blockade influences bone metabolism directly via osteoclastogenesis stimulation. Survival extension of osteoclasts is the main mechanism. Cytokines, interleukines 1 and 6, osteoprotegerin, bone resorption potentiation, osteocytes and osteoblasts apoptosis are other important mechanisms resulting in osteosynthesis inhibiton. AIs also play key role in calcium metabolism. Their action influence calcium absorption in small bowel and renal elimination. It is very similar to estrogens level decrease after menopause leading to postmenopausal osteoporosis (Rizzoli, 2005).
AIs in breast cancer treatment are used as adjuvant therapy – it means after radical surgery in early breast cancer, stages I - III or as palliative therapy of locally advanced or metastatic disease. Standard duration of adjuvant hormonal therapy is now 5 years. Recently

published results of large international multicenter clinical studies (including more than 15 000 pacients) such as ATAC (Howell et al., 2005) and BIG (Coates et al., 2007) have shown that adjuvant hormonal therapy using AIs and lasting 5 years is more effective than adjuvant therapy using selective estrogen receptor modulators (SERMs), mainly tamoxifen. Substudies of these and many other similar studies dealing with bone mineral density (BMD) in early breast cancer patients on adjuvant hormonal therapy are consistently showing higher decrease of BMD during treatment with AIs than that with tamoxifen. This is the reason why regular BMD measurements at the beginning and during AIs adjuvant therapy were implemented into new recommendations for early breast cancer therapy published in July 2007 as results of consensus of panel of most important international leaders in the field during 10th St Gallen Conference (Goldhirsch et al., 2007).

2.2 Patients and methods

We started regular bone mineral density (BMD) measurement of postmenopausal early breast cancer patients treated either with aromatase inhibitors (AIs) or tamoxifen in St. Elisabeth Cancer Institute on September 2005. The most important goal of our study was to determine bone mineral density decrease in early breast cancer patients treated with (AIs). We measured BMD at the begining of treatment and during therapy (after one year or two depending on initial results) with AIs and a group of patients who have their hormonal therapy switched from tamoxifen do AIs for different reasons (intolerance or toxicity).

As an comparative groups we measured BMD in group of early breast cancer patients treated with tamoxifen and patients after finished hormonal therapy without any anticaner therapy, only on regular follow-up. The study is sitll active, in this preliminary evaluation we analysed group of 263 consecutive patients with early breast cancer, 42 on active AIs therapy - 22 on letrozole on oral daily dose 2,5 mg, 20 on anastrozole on oral daily dose 1 mg, 72 patients with „switched" therapy from tamoxifen to AIs, 69 on active tamoxife therapy on oral daily dose 20 mg and 80 patients just on follow-up after finishing active hormonal treatment.

In all our patients the BMD measurement was performed on total body densitometer Hologic Explorer. We measured and evaluated region of proximal femur and L spine. In cases of degenerative changes which overestimated results we measured and evaluated the region of forearm. For comparisons we evaluated T score. All patients included in our study have measured height, weight, assessed age, duration of menopause, hormonal replacement therapy and history of other risk factors and previous fractures. All patients have measured calcium blood level. We also measured markers of bone turnover, CTX (CrossLaps - C telopeptide of alfa chain 2(I) colagen) as marker of osteoporosis measured by ELISA method and isoensyme of ALP as marker of osteoproduction. In patients with BMD results on levels of osteoporosis we made differential diagnostic examinations to exclude secondary osteoporosis. This is important especially in patients with breast cancer to exclude bone marrow metastases, which are most frequent sites of generalised disease. We used cancer markers, RTG, CT, MRI or bone scan - sceletal gamagraphy

For the statistical analysis we used standard methods of descriptive statistics, test of data independence and multiple regression was used to verify influence of separate factors especially to exclude possible secondary influences in case of interactive correlation among parameters.

2.3 Results

From the whole study group of 263 postmenopausal early breast cancer patients, 114 patients in the group treated with AIs (72 of them switched from previous tamoxifen to AIs), 69 on tamoxifen therapy and 80 patients without hormonal therapy only on follow-up after finishing hormonal treatment (figure 1).

Aromatase Inhibitors (AIs)	
Anastrozole	22
Letrozole	20
A/L (after first line TMX)	72
AIs total	**114**
Tamoxifen	**69**
Follow-up - no therapy	**80**

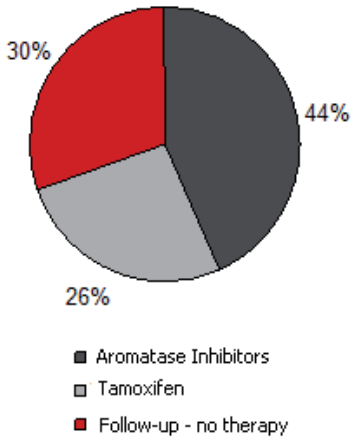


Fig. 1. Patients Characteristics

We found normal BMD only in 13,31% among all evaluated patients, 43,35% of the whole analysed patients had BMD rate in levels of osteoporosis. Analysing the localisations of measured osteoporosis we found this in 5,25% in proximal femur, 63,1% in L spine and 31,58% in region of the forearm (figure 2) – those were patients with deformations or degenerative changes in region of spine, which overestimated the results.

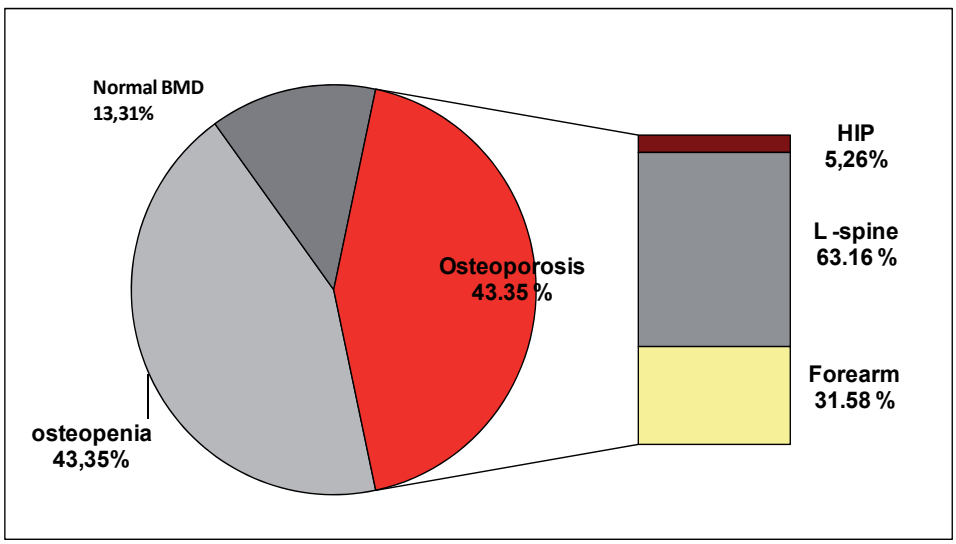


Fig. 2. BMD in Patients with Breast Cancer

Median of age of the whole group of patients was 61 years. The BMD loss to levels of osteoporosis was found in group of patients under 50 years of age in 26%, where 50% of them had osteoporosis in region of L spine, 38% in region of proximal femur and only 13% in region of forearm. The rate of osteoporosis was higher in the group of patients older than 70 years - 73% and most of them had osteoporosis in the region of forearm – 49%. The region of L spine was overestimated by degenerative and deformative changes in this age group of patients. Group of patients in age between 50 to 70 years had BMD levels of osteoporosis in 34%, most frequently in region of L spine – 80%. These findings are in correlation with many clinical studies confirming rising incidence of osteoporosis with rising age. We confirm influence of menopause duration on osteoporosis as well as negative correlation of weight and osteoporosis in our study. All this findings are in consensus with literature data.

We also analysed impact of therapy on BMD loss. In the group of patients with AI therapy BMD loss to level of osteoporosis was diagnosed in 43,86% and normal BMD had 13,16% of patients, in the group with tamoxifen therapy the rate of osteoporosis was 30,43% and normal BMD had 18,84% of patients, in the group on follow-up without hormonal therapy the rate of osteoporosis was 53,75% and normal BMD had 8,7% of patients. The correlation between BMD loss and hormonal therapy was not proven statistically significant despite trend of tamoxifen protective effect on BMD maintenance. This was not statistically significant - (p=0,0610). In subanalysis, where we correlate BMD loss only in subgroup of patients treated by AIs at least one year and patients treated less than 1 year or just on follow-up without hormonal therapy (figure 3), the difference was statistically significant. The rate of BMD loss to level of osteoporosis was 53,13% in the first group and only 40,2% in the letter and normal BMD rate was only 3,13% in the first group versus 16,58% in second one - (p=0,0150).

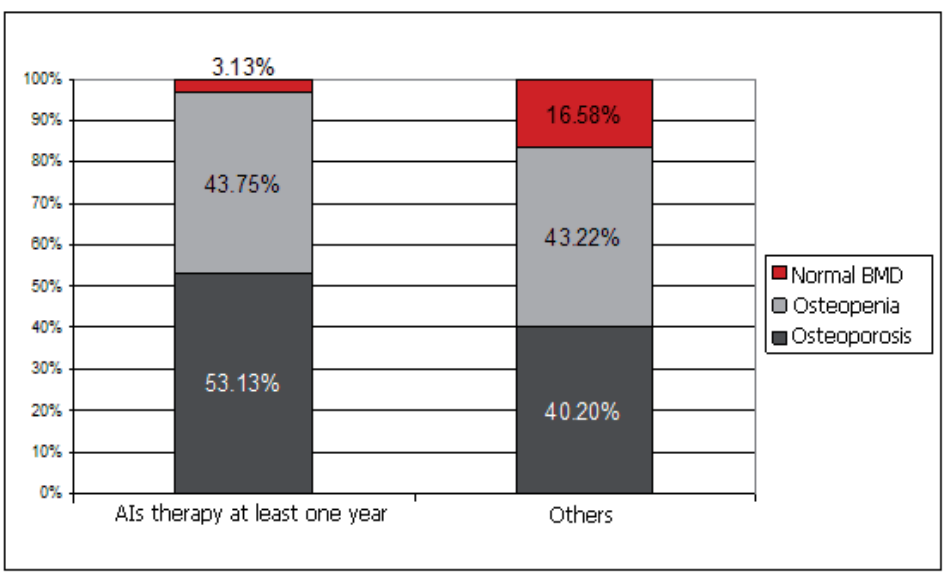
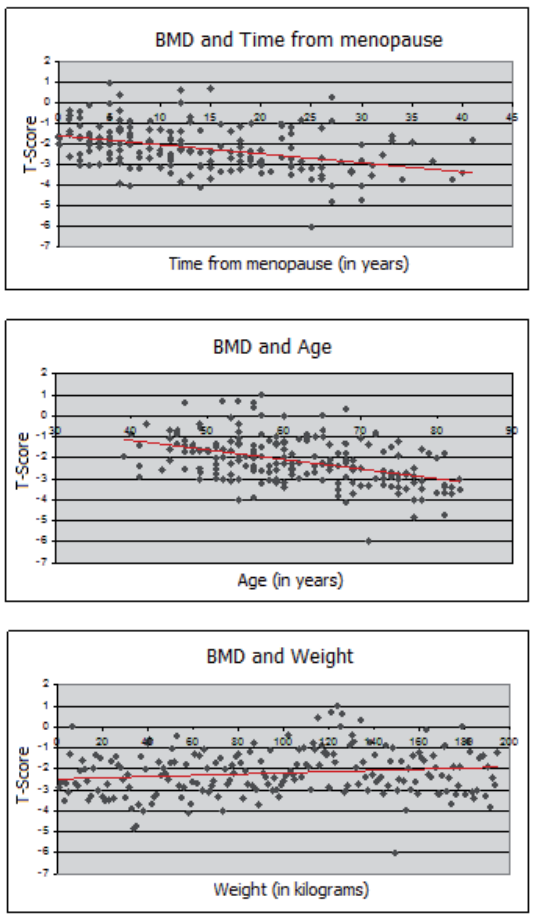


Fig. 3. BMD in Patients with Aromatase Inhibitors Therapy and Others

We analysed other risk factors and we found highest rate of patients with diabetes mellitus among those risk factors (33 patients) but we did not confirm statistical significant influence of diabetes mellitus on BMD loss - (p=0,816).
Correlation of BMD loss and increase levels of CTX as a marker of bone resorption was not confirmed in our study.
We tested all above mentioned risk factors statistically also (figure 4) using method of multiple linear regression to eliminate potential secondary influences in cross interactions among factors. Correlations BMD level to age (p<0,0001 and weight (p<0,0001) were confirmed by multiple linear regression. Borderline statistical significance was shown in correlation to AIs therapy (=0,0476). The influence of time from menopause (p=0,3410) seemed to be secondary regarding to high correlation to age of patients (r=0,89, p< 0,0001).



Correlation coefficients:

(BMD,Age) = -0.44 (p<0.001)
(BMD,Weight) = 0.43 (p<0.001)
(BMD,Time from menopause) = -0.39 (p<0.001)

Fig. 4. Correlation of BMD with Age, Weight and Time from Menopause

The rate of pathological fractures was analysed also (figure 5). The most frequent incidence was in group of patients with osteoporosis. Wrist fracture was found in 10 patients and 5 had fractures in region of L spine. In the group of patients with BMD on level of osteopenia, 5 patients had pathological fractures, 3 of them were wrist fractures and 2 in region of L spine. There were only 2 pathological fractures in patients with normal BMD levels, both were wrist fractures. The whole group of patients we considered to be too small to make statistical analysis of risk factors of pathological fractures.

The last was the analysis of influence of antiresorptive therapy on BMD changes (figure 6). The analysis seemed to be preliminary as in the control group (control BMD measurement after 1 year of duration of antiresorptive therapy) were only 53 patients. This did not allow us to make relevant statistical analysis, although we found trend toward protective effect of antiresorptive therapy in this group of patients.

Osteoporosis	
Wrist	10
Spine	5
Hip	0
Osteopenia	
Wrist	3
Spine	2
Hip	0
Normal BMD	
Wrist	2
Spine	0
Hip	0

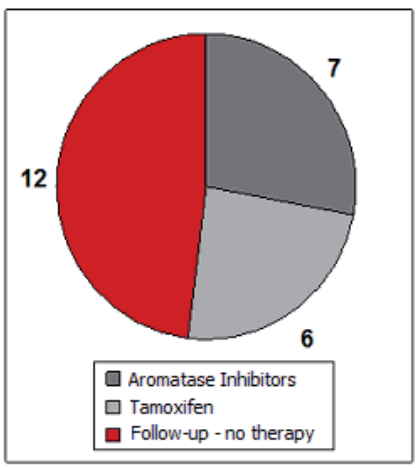


Fig. 5. Patological Fractures Rate

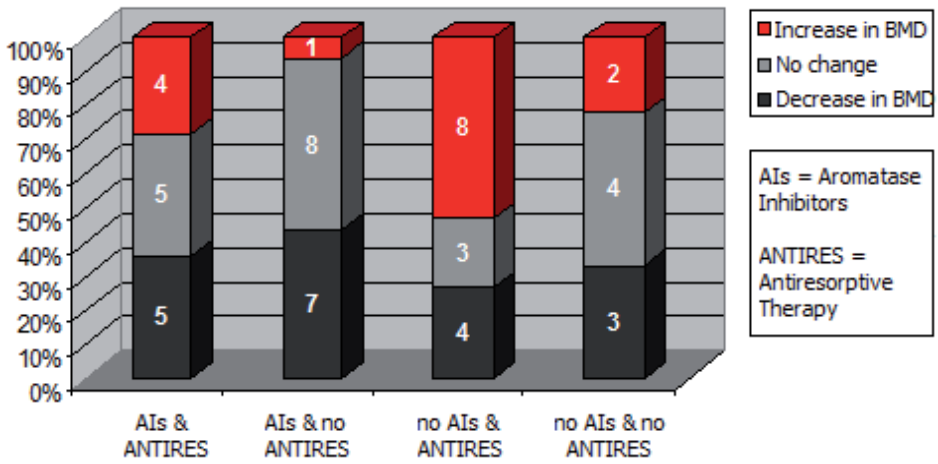


Fig. 6. Impact of Antiresorptive Therapy on BMD in patients with Breast Cancer

2.4 Discussion

The AIs are new standard in adjuvant hormonal therapy of early breast cancer postmenopausal patients. As the results of many large international multicentre clinical trials are more mature and results of substudies focused on BMD loss more and more consistant, new standards for BMD examination are evolving. The prognosis of early breast cancer patients is continually improving. BMD loss means increasing risk of osteoporosis and it means increasing risk of pathological fractures. There are many risks factors for this group of patients - age, postmenopausal status, breast cancer, AIs therapy. Adjuvant hormonal therapy is one of the most important factors leading to significant improvement in patient survival and the same important is quality of life which may be markedly decreased by pathological fractures from osteoporosis.

Identification of all risk factors of origin and progression of osteoporosis as well as exact examination procedures to find them is as important as prevention and therapy of BMD loss. Generally confirmed risk factors for pathological fractures of osteoporosis in breast cancer patients are:

AIs therapy
T-score < 1. 5
Age > 65 years
Low body mass index (BMI < 20 kg/m^2)
Family history of hip fracture
Personal history of fracture from osteoporosis after age of 50
Oral corticosteroid therapy lasting > 6 months
Smoking (in present or in past)

Table 1. Risk factors for pathological fractures of osteoporosis in breast cancer patients

In multicenter international clinical trial „ATAC", where the postmenopausal early breast cancer patients were randomised (final design) to AI anastrozole (A) versus tamoxifen (T) showed that after 5 years of therapy (Howell et al., 2005) there were significantly more bone fractures on arm A (11% versus 7%; p < 0. 001). In clinical trial „BIG 1-98" the same postmenopausal early breast cancer patients were randomised to AI letrozole (L) versus tamoxifen (T). With median of follow-up of 26 months (Thurliman et al., 2005) there were significantly more bone fractures on arm L (5.7% versus 4.0%; p < 0. 001). Very similar results were reached in the clinical study „IES" where the postmenopausal early breast cancer patients were randomised to AI exemestane (E) versus tamoxifen (T) and with median of follow-up ~ 56 months (Coombes et al., 2007) there were significantly more bone fractures on arm E (7% versus 4. 9%; p = 0. 003). In combined clinical study „ABCSG-8 and ARNO 95" the patients were „switched" after anastrozole (A) therapy to tamoxifen (T) vs continuing T therapy. With median of follow-up of 28 months (Jakesz et al., 2005) there was similar significant difference against arm A (2% versus 1%; p = 0. 015). In the clinical study „MA.17" were the patients after 5 years on tamoxifen (T) therapy randomised to „switch" to anastrozole (A) versus only follow-up without

hormonal treatment. With median of follow-up of 30 months (Goss et al., 2005), there were more patients with newly diagnostic osteoporosis on arm A (8. 1% versus 6. 0%; p = 0. 003), and more bone fractures (5. 6% versus 4. 6%, this difference was however not statistically significant p = 0. 25).
In comparison of patients on AI anastrozole (A) therapy from the clinical study „ATAC" to their healthy counterparts matched in age, postmenopausal status, with osteopenia, the incidence of bone fractures were nearly doubled.
We confirmed the prognostic importance of age, duration of menopause, AIs treatment in comparison to tamoxifen treatment or no therapy in follow-up group in our clinical observation. All these results are in concordance with world scientific literature.
According to WHO and NOF (National Osteoporotic Foundation) guidelines is the value of T-score in BMD measurement critical in distribution to normal BMD (T-score ≥ - 1. 0), osteopenia (T-score between – 1. 0 and – 2. 5) and osteoporosis (T-score ≤ - 2.5) (Kanis et al., 2008). According to international general guidelines is this classification universally accepted and it is recognised that with decreasing BMD level the risk of pathological bone fractures is rising. That is why the results and observations of the clinical study NORA (National Osteoporosis Risk Assessment) are so interesting. They observed > 200 000 healthy postmenopausal women and found that 82% pathological bone fractures happened in women with T-score > - 2. 5, which means that they did no have osteoporosis and 52% fractures were in women with osteopenia (T-score – 1. 0 to – 2.5).
All these results and findings confirm the importance of BMD measurement before AIs therapy initiation and importance of preventive measurements as components of adjuvant AIs therapy as well. Calcium and vitamin D supplementation and appropriate physical activity are standard components of these recommendations (Goldhirsch et al., 2007). Preventive bisphosphonates application is being evaluated in many running clinical studies. Especially zoledronic acid is showing excellent results and it seems to be incorporated into standard combination with AIs in adjuvant therapy of postmenopausal early breast cancer patients very soon as osteoporosis and bone fracture prevention (Gnant et al., 2007).
There was observed protective effect against bone loss, longer period to bone metastases occurence and suspected direct anticancer effect as well. These results will probably lead very soon to change today ´s standards and bisphosphonates will be used together with AIs in adjuvant therapy of early breast cancer patients (Gnant et al., 2007).
The influence of antiresorptive therapy on BMD was part of our study as well. This analysis is difficult to interpret as our control group (control BMD measurement after 1 year of duration of antiresorptive therapy) was very small (only 53 patients) and median of follow-up very short. This did not allow us to make relevant statistical analysis but we found trend toward protective effect of antiresorptive therapy in this group of patients.
We did not confirm correlation of BMD decrease and CTX elevation. Probably the reason was small analysed group of patients and low specificity of CTX as osteoporosis marker (S.Špánik & B. Špániková, 2010).

2.5 Conclusions and future directions

The most important goal of our study was to confirm the importance of BMD measurement and evaluation in group of postmenopausal early breast cancer patients on AIs therapy. Even the study group is not very large, all the patients are from single

institute and we have planned to follow-up them throughout the AIs therapy and thereafter. Preliminary analysis of our data confirmed significant BMD loss in this group of patients. The AIs therapy influence on BMD loss was statistically significant after one year of therapy. For more valid data we need more patients and longer time of follow-up. Our plan is to continue in evaluation of influence of antiresorptive therapy on BMD as we observed trend of protection of BMD. Evaluation of importance of BMD loss for increase risk of pathological bone fractures also needs more patients and longer time of follow-up (Hadji et al., 2011).

Our observational study confirmed importance of BMD measurement and evaluation in postmenopausal early breast cancer patients on AIs therapy. This is in concordance with new recommendations for early breast cancer therapy published in July 2007 as a result of consensus conference (10th St Gallen Conference) and other important international guidelines.

3. Testicular cancer

3.1 Introduction

Testicular cancer (TC) is still being serious disease although when the patients are correctly diagnosed and treated the cure rate is about 90%. Testicular cancer make about 1% of malignant tumors in men, the incidence in recent years is going up. The incidence in Slovak Republic in 2003 was 7,3/100000 men and during last 30 years has increased almost 5-times.TC appear mostly in men from 20 to 40 years of lilfe. (D. Ondruš & M. Ondrušová, 2008). According to international classification more than 95% of TC are germ cell tumors (GCT), which are classified into two major subgroups: seminoma and non-seminoma GCT. Nonseminomatous GCT comprises approximately 50% of all GCT. Most tumors are mixed, consisting of two or more cell types (embryonal carcinoma, choriocarcinoma, yolk sac tumor, teratoma or their mixtures). The rest of testicular tumors are rare - Leydig cell tumors, Sertoli cell tumors, granulosa cell tumors, gonadoblastoma, sarcomas, lymphomas and others).

The diagnosis of GCT is based on clinical picture – painless testicular mass, symptoms of epididymitis or orchitis, less frequently occurs testicular pain.

For pretreatment staging we use ultrasound, computed tomography (CT) of chest, abdomen and pelvis and serum tumor markers (alfa-fetoprotein, human chorionic gonadotropin, lactate dehydrogenase).

The standard therapeutic procedure is surgery, radical orchciectomy and retroperitoneal lymph node dissection (according to histological type and stage of the disease). Other therapeutic options are radiotherapy and chemotherapy (again according to histological type and stage of the disease).

During recent decades the survival rate of patients with testicular cancer or germ cell tumors (GCT) has substantially improved. Consequently the long-term side effects of treatment of GCT have gained attention, including accelerated bone loss leading to increased risk of osteoporosis. Treatment-related bone loss is well recognized in breast and prostate cancer, but there has been little information in long-term survivors from other tumors (Marcus et al., 2008).

We have a large group of GCT patients in our registry at the Department of Urology of St. Elisabeth Cancer Institute with a long duration follow-up. It is already known that

androgens influence bone modelling and remodelling acting on osteoblasts, osteocytes and pluripotent stem cells through androgen receptors. They also act indirectly via estrogen receptors. The combined influence of androgens and estrogens is even stronger. We suppose that patients after unilateral orchiectomy (OE) or bilateral orchiectomy and consecutive radiotherapy and/or chemotherapy should have lower levels of testosterone. Literature sources on this issue are scarce and conflicting. There are numerous animal studies proving the effect of androgens on bone and also proving much stronger effect of androgen and estrogen combination (Ondruš et al., 2007).

3.2 Patients and methods

Our aim was to determine BMD and serum bone turnover markers in survivors from GCT. We included 719 patients with GCT into the study. We measured BMD in GCT patients from 2005.

BMD was measured by dual-energy X-ray absorptiometry using osteodenzitometer Holgic Discovery in the lumbar spine and hips. BMD was classified as osteopenia (T score ranging from –2, 5 to –1.0) and osteoporosis (T score less than –2. 5). Latter according to WHO recommendation for men under 50 years of age we use Z score (comparison of detected BMD to healthy bone of comparable age group).

C-terminal cross-linked telopeptides of type I collagen (CTX) were measured using Enzyme-Linked Immunoabsorbent Assay (ELISA). Additionally serum total testosterone was measured.

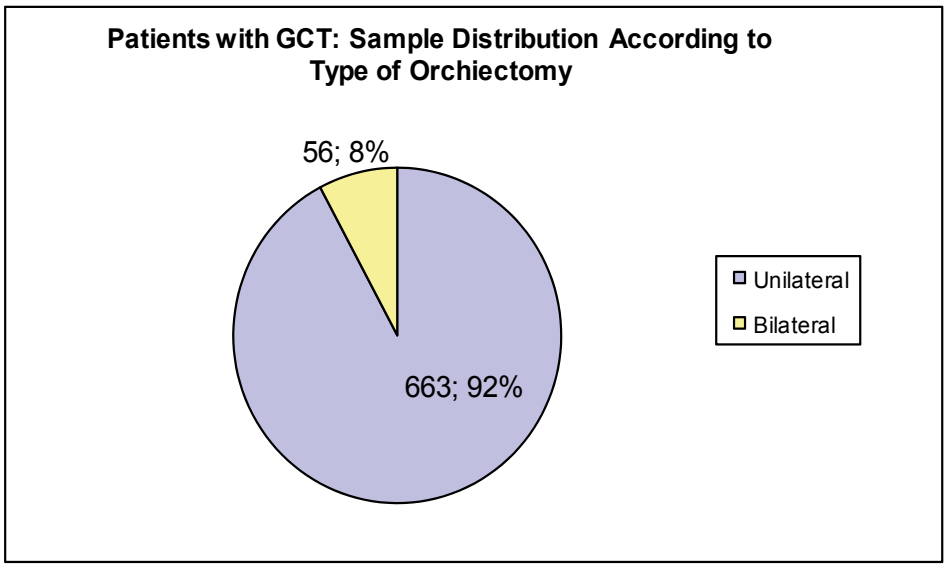
Comparison was made with matched healthy control group from Ministery of health registry. Relationships between baseline characteristics (age, treatment type and time from orchiectomy) and BMD were assessed using univariate and multivariate analysis tools.

The data was evaluated using Microsoft Excel 2003 software and its built-in statistical functions and data analysis tools. We used standard uni-, bi- and multivariate statistical methods as appropriate throughout data analysis. Association between two nominal variables was tested using the Chi-squared test of independence. Association between an interval variable and a nominal one was tested using ANOVA or Kruskal-Wallis test, depending on the distribution of the interval variable; in case of a dichotomous variable, t-test was applied instead of ANOVA and Mann-Whitney test instead of Kruskal-Wallis test. A multiple linear regression was performed to test for association between an dependent interval variable and several predicting interval variables (Mardiak et al., 2007)

3.3 Results

We included 719 patients into the study (21 – 76 yrs old, median: 39 yrs) who were treated for GCT since 1982. In this group, 663 pts (92%) were treated by unilateral orchiectomy (OE) and 56 pts (8%) by bilateral OE. The further treatment was radiotherapy of retroperitoneal lymph nodes (RPLND) in 124 pts (17%), chemotherapy in 405 pts (57%), radiotherapy and chemotherapy in in 16 pts (2%), the rest 174 pts (24%) did not receive any adjuvant therapy (fig. 7). Median time since OE was 6. 5 yrs, average time was 8. 1 yrs.

We have proved a significant difference between BMD patients with GCT compared to the healthy population ($p<0.0001$) with more osteopenia and osteoporosis in GCT patients.



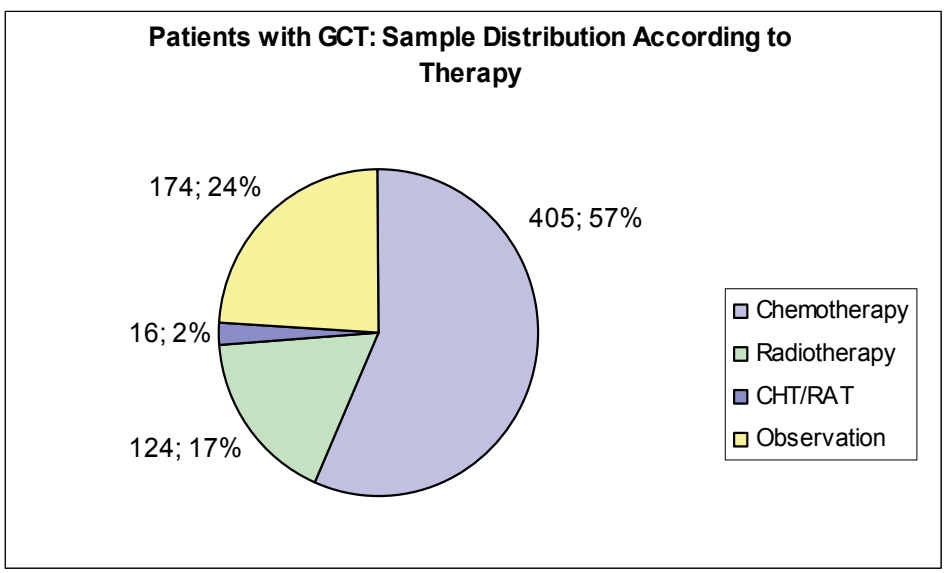


Fig. 7. Patients characteristics

We have made comparisons between the group of GCT patients and the healthy match control group according to type of surgery and subsequent therapy (fig. 8)

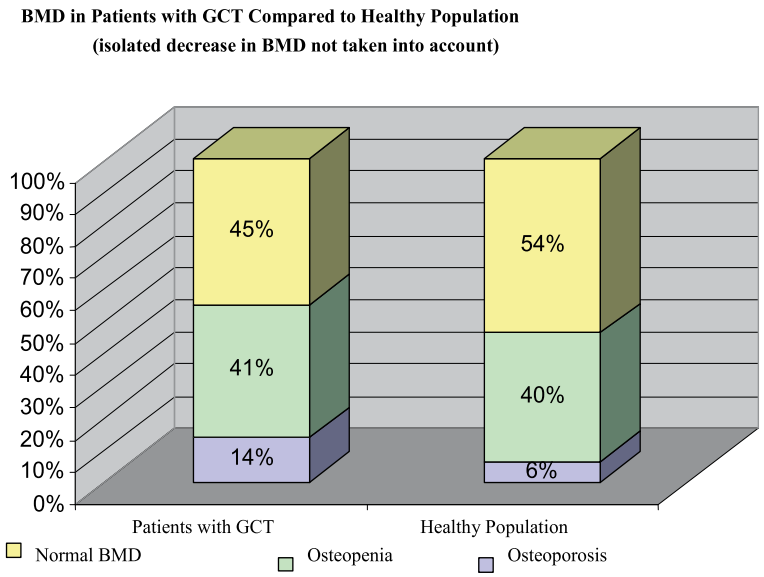


Fig. 8. Comparison of GCT group to healthy match control group

We made comparison of BMD and the type of orchiectomy (fg. 9)

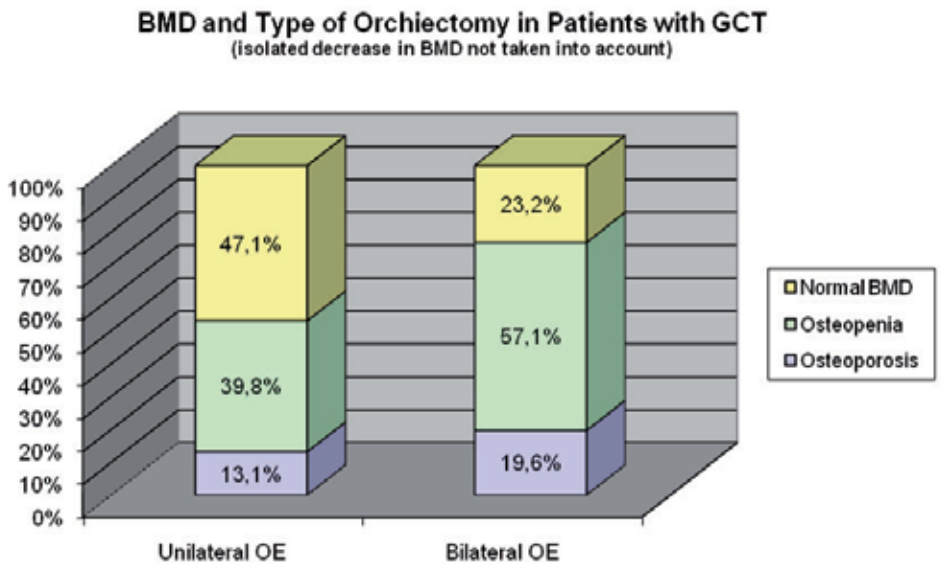


Fig. 9. Comparison of BMD and type of OE

Comparison was made between the subgroup of patients with unilateral orchiectomy and a the subgroup of those treated with bilateral orchiectomy. While the incidence of osteoporosis has not proved to be significantly different in the two subgroups (p=0.1725), patients treated with bilateral OE have significantly higher incidence of osteopenia (p=0.0116).

We also made comparisons of BMD of patients with GCT and different types of OE to healthy match control group (fig. 10)

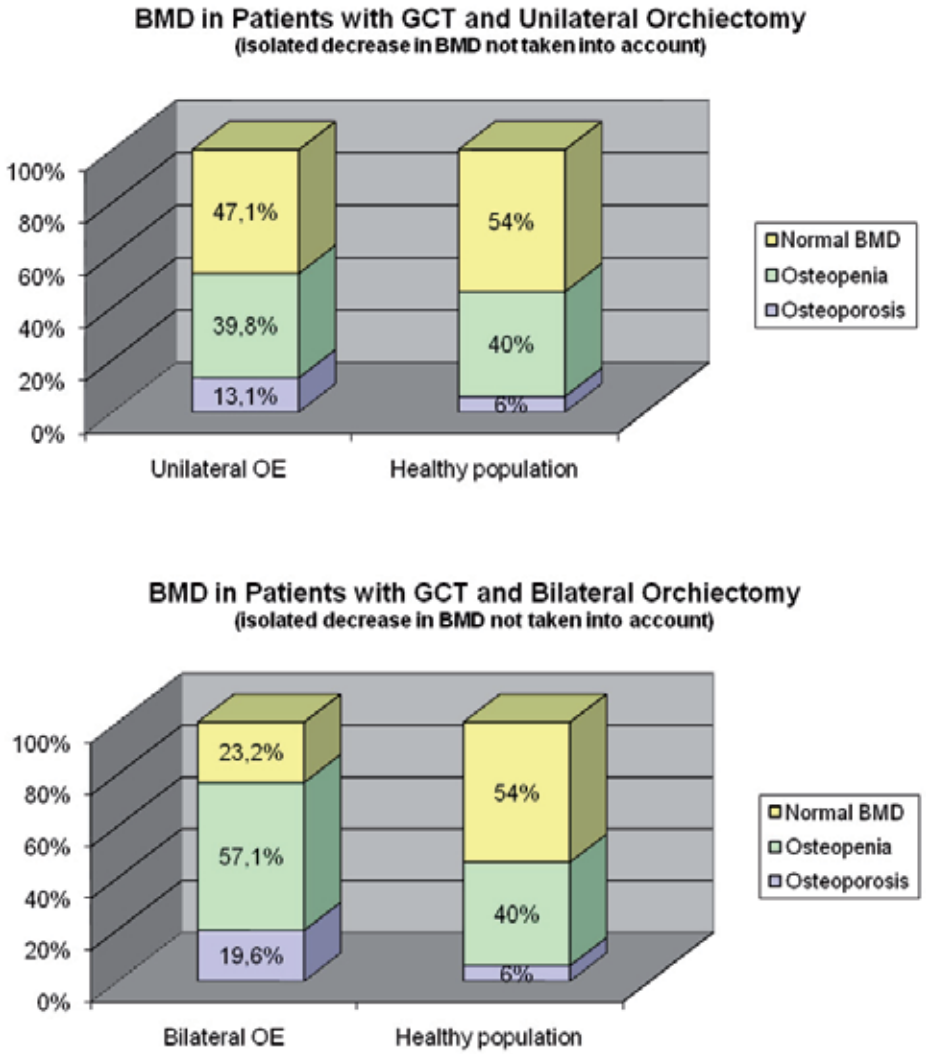


Fig. 10. Comparisons of BMD of patients with GCT and different types of OE to the healthy match control group

In a separate comparison of the subgroup of patients treated with bilateral orchiectomy to the healthy population we have concluded not only a significantly higher incidence of osteoporosis in the former ($p<0.0001$), but also a significantly higher incidence of osteopenia in those patients treated with bilateral OE ($p=0.0114$). Patients treated with unilateral OE compared to the healthy population have a significantly higher incidence of osteoporosis ($p<0.0001$) We also made comparisons of BMD according to time from primary therapy

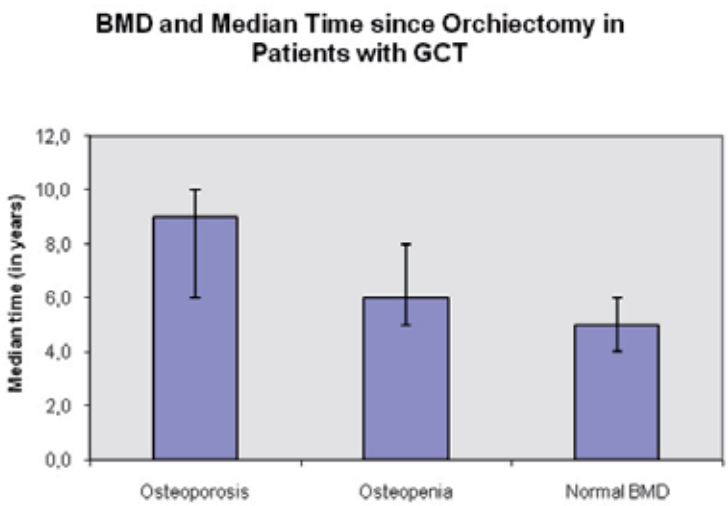


Fig. 11. BMD and median time since OE

All the most important results are summarized in table 2.

	Total	Osteoporosis	Osteopenia	Normal BMD	P value
Number of patients:	719	98 / 14%	296 / 41%	325 / 45%	
Treatment:					
Unilateral OE	663	87 / 13%	264 / 40%	312 / 47%	
Bilateral OE	56	11 / 20%	32 / 57%	13 / 23%	
Chemotherapy	405	48 / 12%	172 / 42%	185 / 46%	
Radiotherapy	124	19 / 15%	48 / 39%	57 / 46%	
Chemo- and Radiotherapy	16	3 / 19%	10 / 63%	3 / 19%	
Characteristics (Risk Factors):					
Fractures	98	10 / 10%	47 / 48%	41 / 42%	
Demographics:					
Median age		43,0	39,0	37,0	(<0.0001)
Average age		43,8	39,5	37,7	(<0.0001)
Baseline Characteristics (medians):					
Time since OE	702	9,0	6,0	5,0	(0.0049)
Testosterone (nmol/l)	478	16,8	16,6	16,3	(0.6887)
Free Testosterone (pg/ml)	132	7,0	8,3	8,3	(0.3526)
CTX (scale 1: ng/ml)	137	0,4	0,5	0,4	(0.5545)
CTX (scale 2: pM)	287	3341,0	4175,5	3941,5	(0.2101)
LH		4,6	4,6	4,6	(0.3602)

Table 2. Patients with GST – characteristics and results

3.4 Conclusions and future directions

- We have proved a significant difference between BMD patients with GCT and healthy population ($p<0.0001$).
- The incidence of osteoporosis is significantly higher in patients with GCT compared to the healthy population ($p<0.0001$).
- While the incidence of osteoporosis has not proved to be significantly different in the two subgroups ($p=0.1725$), patients treated with bilateral OE have significantly higher incidence of osteopenia ($p=0.0116$).
- Comparing the minimum T-score between the two subgroups, we have concluded significantly lower median T-score in patients with bilateral OE compared to those treated with unilateral OE ($p=0.0148$).
- We have come to the conclusion of no significant association between BMD and type of therapy in patients with GCT ($p=0.287$).
- There is no significant association between BMD and CTX level ($p=0.1600$).
- Correlation between T-score and testosterone also free testosterone level in patients with GCT, the correlation coefficient of 0.0881, did not prove as significant ($p=0.3263$).

We did not find any significant differences between GCT and matched control data regarding the incidence of osteopenia and bone turnover marker, but the incidence of osteoporosis was considerably higher in GCT patients. The incidence of osteopororsis appeared to increase with age and to slightly correlate with time since OE, particularly after 10 years following OE. Type of therapy did not prove to have significant impact on the appearance of osteoporosis. Serum testosterone level did not correlate with BMD. We recommend BMD measurement and evaluation in GCT patients after therapy (Ondrušová et al., 2009).

4. Bone mineral density in thyroid cancer (TC) patients on suppresive therapy after total thyroidectomy

4.1 Introduction

Thyroid cancer (TC) is another type of cancer which should be associated with decrease of BMD after total thyreoidectomy and subsequent suppressive therapy. Although TC comprises only 1. 1 – 1. 9% of all cancers it is the most frequent endocrine tumor making around 90% of them. It is 3-times more frequent in women than in men. The most frequent types are well-differentiated cancers – papillary (80 – 85%) and less frequent follicular (5 – 10%) thyroid carcinoma.

The standard therapy of thyroid carcinomas (TC) consists of total or nearly total thyroidectomy. Then the whole-life substitution therapy by oral thyroxine (T_4) is given. Another goal of this therapy is to suppress serum thyroid stimulating hormone (TSH) to prevent the growth factor-like effect of TSH on well-differentiated TC cells. The recommendation is to administer supraphysiologic amounts of oral T_4. This leads to hyperthyroidism, which is subclinical. The influence of thyroid hormones on bone tissue is well known (Altabas et al., 2007). In hyperthyreosis the acitivty of osteoblasts and osteoclasts is increased, but the influence of osteoclasts is dominating leading to bone resorption and osteoporosis. The longstanding subclinical hyperthyroidism may result in

increased bone turnover and decreased bone mineral density (BMD). The aim of the study was to assign the damage of bone metabolism in TC patients.

4.2 Patients and methods

Bone mineral density (BMD) was measured by dual energy photon x-ray absorptiometry BMD using osteodenzitometer Holgic Discovery in the lumbar spine and hips. In cases with arteficialy increased bone density caused by degenerative bone changes we measured BMD also in forearm. BMD was classified using T score in postmenopausal women and more than 50 years old men . We classified Z score in premenopausal women and younger than 50 years men. C-terminal cross -linked telopeptides of type I collagen (CTX) were measured using ELISA. Additionally serum TSH and fT_4 (free T_4) were measured. Relationships between baseline characteristics (age, menopausal status in women, TSH levels and duration of substitutional therapy) and BMD were assessed using univariate and multivariate analysis tools.

Association between two nominal variables was tested using the Chi-squared test of independence. Association between an interval variable and a nominal one was tested using ANOVA or Kruskal-Wallis test, depending on the distribution of the interval variable; in case of a dichotomous variable, t-test was applied instead of ANOVA and Mann-Whitney test instead of Kruskal-Wallis test. A multiple linear regression was performed to test for association between an dependent interval variable and several predicting interval variables.

We analysed BMD data from 165 TC patients after total tyroidectomy on supportive therapy in our study. There were 13 men and 152 women, 94 were postmenpausal (postMP), 44 premenopausal (preMP), in 14 the menopausal sattus was unknown. The mean age was 51 years. Age characteristics are in table 3.

Age characteristics:				
	All	Men	PostMP women	PreMP women
Min	21	29	34	21
Max	78	77	78	55
Mean	53	49	59	39
Median	54	50	58	41
Standard deviation	12.65	14.21	8.25	8.45

Table 3. Age characteristics

4.3 Results

We included 165 TC patients in the study. There were 13 men a 152 women, the mean duration of thyroid suppressive therapy was 7. 2 years (0 – 24 years). The patients exhibit osteporosis in 31%, 39% had osteopenia, whereas 30% had normal BMD (fig. 12).

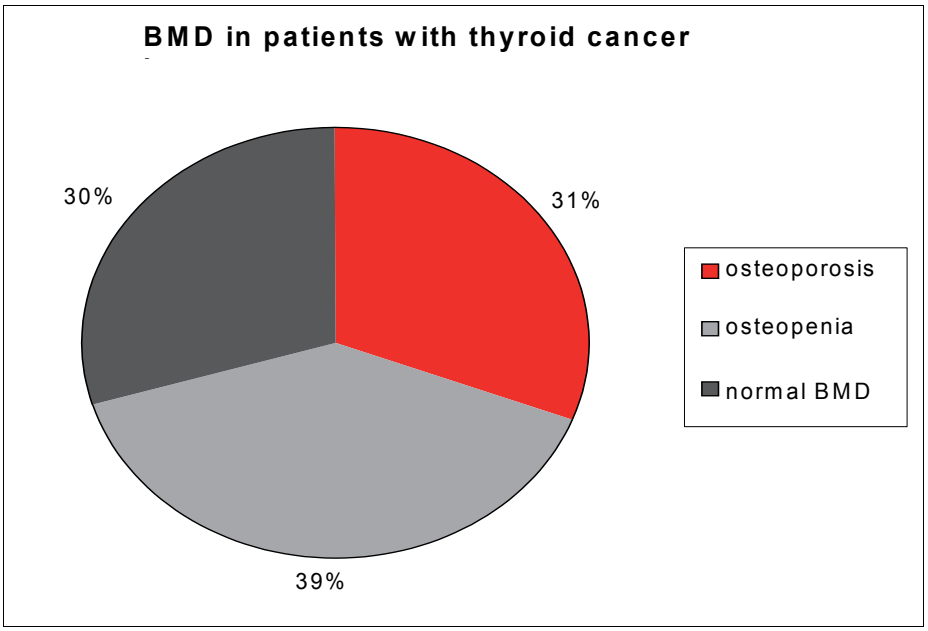


Fig. 12. BMD in TC patients

The BMD changes were localised in different sites (fig. 13)

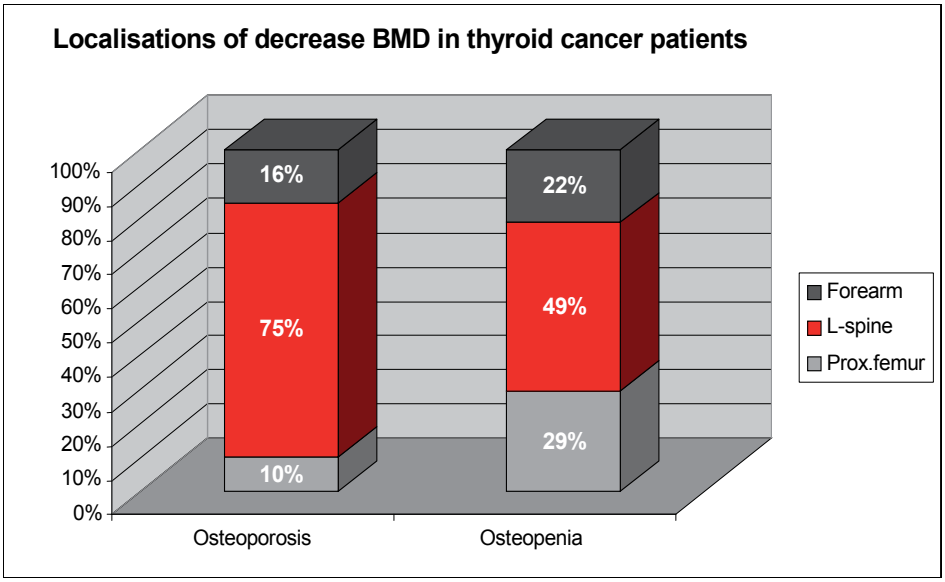
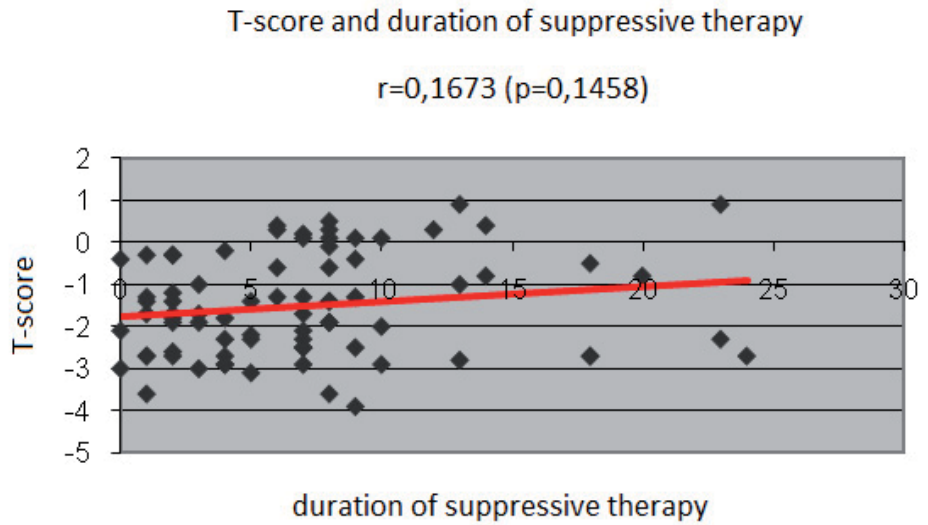


Fig. 13. Localisations of BMD decresae in TC paients

The incidence of osteoporosis appeared to increase with age but did not correlated with duration of thyroid suppressive therapy (fig. 14)



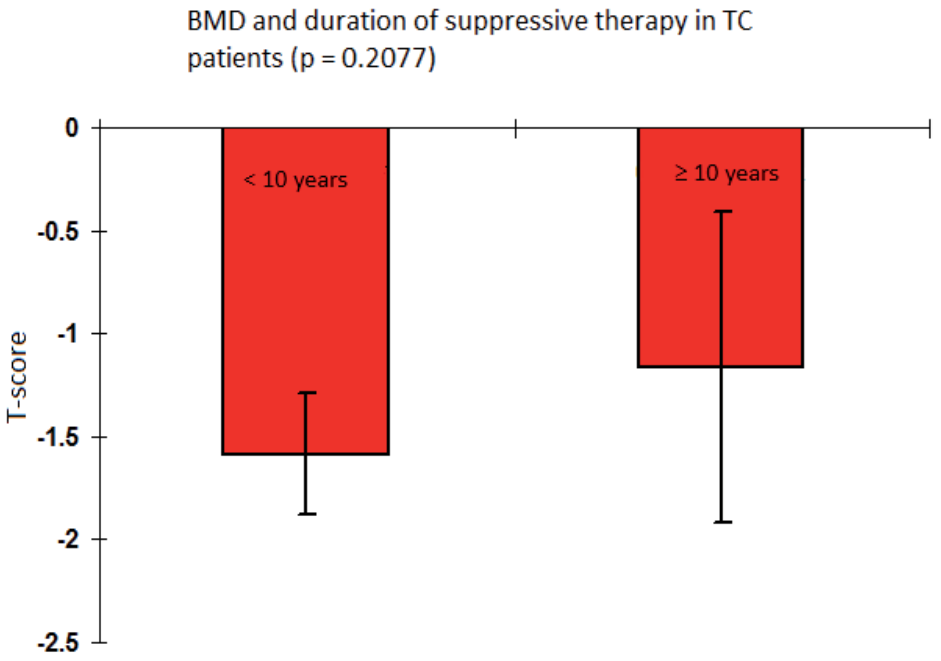


Fig. 14. Duration of suppressive therapy and BMD

We confiirmed higher incidence of osteoporosis in postMP women and in the small subgroup of postMP women on antiporotic therapy we confirmed efficacy of this therapy (fig.15). But the gorup was very small and we cannot make any conclusuions.

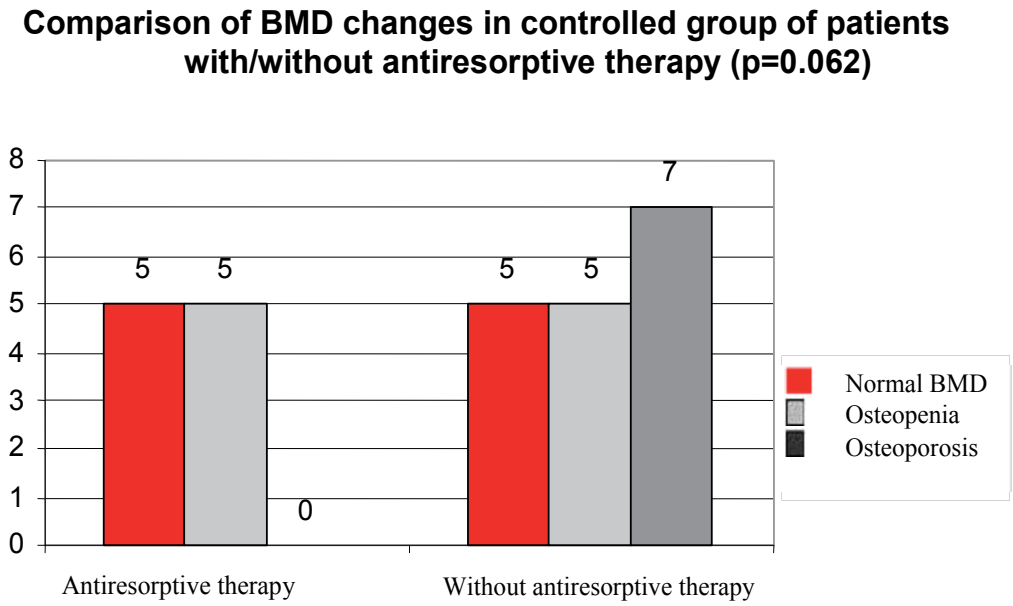


Fig. 15. Comparison of BMD changes in controlled group with/without antiresorpitve therapy

We confirmed 10 pathological fractures in our study (tab. 4)

Fractures
10 pathological fractures
Site • wrist 3 • spine 3 • others 4

Table 4. Pathological fractures in TC patients

The pathological fractures were in correlation with decreased BMD to osteopenia or osteoporosis (fig. 16)

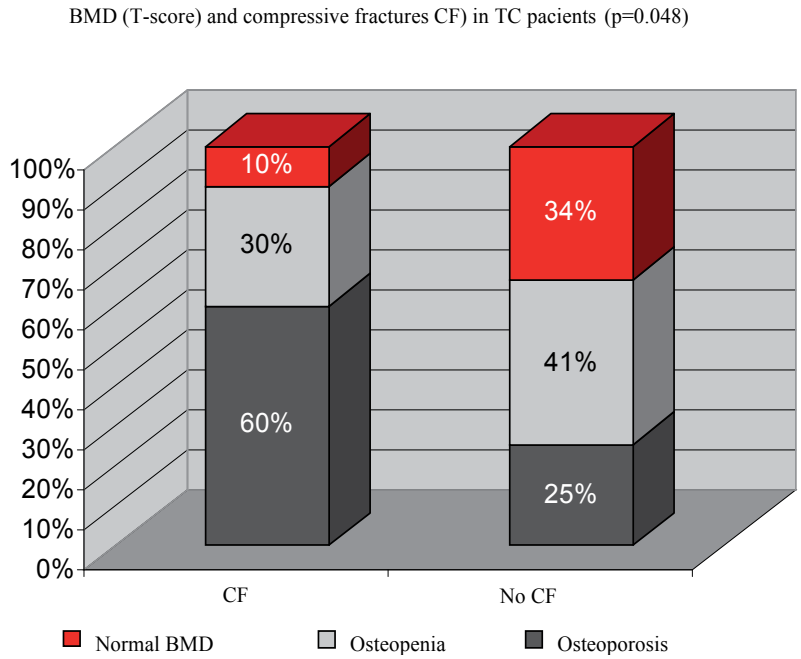


Fig. 16. BMD and compressive fractures in TC patients

4.4 Discussion

Published data on this topic are scarce and conflicting. Some of them did not prove correlation between subclinical hyperthyreosis and decrease in BMD (S. I. Greenspan & F. S. Greenspan, 2005). Others have found, that if suppressive therapy does not suppress the TSH below normal value it does not decrease BMD and does not worsen the prognosis of TC (Biondi & Cooper, 2010). Similar results were published also in the past (Shomon, 1995). Some on the other hand has confirmed that long term suppressive therapy affects bone turnover and bone mineral density in pre and postmenopausal women with TC (Heijekmann et al., 2005). Decrese in BMD as a result of suppressive therapy in TC was confirmed but this effect was ameliorated by preventive substitution of calcium and calcitonin (Mikosch et al., 2006). In this metaanalysis of 8 studies the influence of thyreoidal suppression on BMD in postmenopausal women was confirmed. It was not confirmed in men and premenopausal women. The limitation was a substantial inhomogeneity of patients groups and uneveness in calcium supplementation.

4.5 Conclusions and future directions

The long term survivors from TC after total thyroidectomy on thyroid suppressive therapy have higher risk of osteoporosis and therefore we recommend BMD testing and appropriate measures according to results. The BMD decrease may be a risk factor for pathological fractures and as the TC has very good prognosis, it does matter (B. Špániková.& S. Špánik, 2011)

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Part 5

Pediatric Issues in Osteoporosis

19

Osteoporosis in Pediatric Patients and Its Clinical Management

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1. Introduction

The increase in longevity achieved at present, the population has determined a striking increase in the prevalence of certain diseases and in other cases, the emergence of new forms of illness and during different life stages (1).

Osteoporosis is defined as a decrease in bone mass associated with the deterioration of bone tissue architecture and increased fracture risk, has become a serious public health problem in our society that affects a wide strata of the population age variable, though increasingly common among younger (2). There are two types of osteoporosis, primary and secondary. Primary osteoporosis is rare affecting one case per 100,000 subjects. For high school, its frequency is higher, being secondary to diseases or drug therapies. Table 1 shows the main causes that can lead to a primary or secondary osteoporosis.

The bone will undergo changes during growth reaching its peak during the second decade. After the fourth decade, there is progressive increase in bone loss that mainly affects trabecular tissue at both the peripheral and axial. Accordingly, it is during childhood when determining events occur in the development of adequate bone mineralization and bone mass final (3).

It is now accepted that osteoporosis in the adult subject has its origins in childhood. Accordingly, the prevention of it would begin with the empowerment of those factors that promote the acquisition of optimal bone mass development. Now if we take into account the dietary habits and sedentary marking between the current youth population, we can glimpse the high risk of developing the disorder to an increasingly early age (3).

During the first decade of life, the appendicular skeleton is growing faster than the axial. Also, the bone mineralization process starts in utero found strongly influenced by calcium intake during growth. However, calcium requirements vary throughout life, being greatest during the first years of life and in times such as puberty, pregnancy and lactation. Moreover, the loss of bone mass increases with age and accelerates with menopause. In this sense, it is advisable to increase the intake of calcium from the perimenopause.

For pediatric patients, there is a relationship between bone mass and size. However, in periods like puberty and after the pubertal growth spurt can be established called an imbalance between the rate of bone growth rate and increased bone mass resulting from this transient increase in bone fragility (4).

Primary Osteoporosis	
Idiopathic juvenile osteoporosis	Marfan syndrome
Osteogenesis imperfecta	Homocystinuria
Ehler Danlos syndrome	
Bruck syndrome	
Secundary Osteoporosis	
Neuromuscular Disease	**Procesos Crónicos**
Cerebral Palsy	Leucemia
Duchenne Muscular Dystrophy	Fibrosis Quística
Prolonged Immobilization	Malabsorción intestinal
Endocrine Diseases	Talasemia
Hypogonadism	Cirrosis Biliar Primaria
Turner Syndrome	Nefropatías
Growth hormone deficiency	Anorexia Nerviosa
Hyperthyroidism	Trasplantes
hyperprolactinemia	Infección por VIH
Cushing Syndrome	**Yatrogenia**
Congenital Metabolic Disorders	**Corticosteroids**
Gaucher Disease	Metotrexate
	Cyclosporine
	Heparin
	Anticonvulsivants
	Radiotherapy

Table 1. Causes of primary and secondary osteoporosis in children

On the other hand, it is normal that there is a correlation between the stage of pubertal development and BMD at both the peripheral and axial. Among girls after menarche has been a significant increase in BMD. BMD in boys increases after puberty, with a more extended in time because their pubertal development is slower (5).

Genetic factors in turn, are equally important in the development of an adequate peak bone mass. Thus, a correlation in BMD between twins. For their part, black women have greater BMD and thus a lower incidence of osteoporosis when compared to white women. Also found lower BMD among women whose mothers had a post-menopausal status with osteoporosis compared to those other women the same age but without such a history. Accordingly, it should raise the transmission of genetic information is carried out mainly through the mother (6).

Analyzing the etiology of osteoporosis in children are significantly different from those in adulthood. Accordingly, the diagnostic approach would be completely different in children compared to adults. Among the risk factors associated with the occurrence of bone metabolism during childhood are processes that interfere with proper bone mineralization, the absence of positive stimulus of calcium and vitamin D from diet or exercise to obtain adequate bone mass. Other causes are disorders that cause interference in pubertal

development as well as any conditions that cause increased bone loss. Finally, prolonged exposure to certain drugs that induce the development of osteoporosis.
Given the above should be considered the early diagnosis of osteoporosis in pediatric patients who have had one or more fractures are not preceded by trauma or as a result of minor trauma. Moreover, the development of significant angular deformities in the extremities and the presence of a marked kyphosis should guide the clinician to the presence of impaired bone quality.
This review aims to provide guidance on the characteristics of the process of normal and abnormal bone mineralization in the pediatric patient, the main factors involved and the existing prevention strategies.

2. Bone mass concept and assessment of their status

Bone mass is defined as the total amount of bone tissue in the organism including the extracellular matrix ossified. At present, it is accepted that the acquisition of appropriate peak bone mass is essential to prevent osteoporosis later in life. Since this formation and accumulation of bone mass occurs during the first decades of life, control of bone mineralization during childhood is a significant aspect to assess interest. This monitoring should aim to identify children at risk of developing osteopenia. Also, in the general population should implement measures to prevent the onset of the disease promoting lifestyles and measures to increase bone mass (7).
The development of an adequate level of bone mass is partly dependent on nutritional factors, so it is necessary to maintain an adequate nutrient supply during the growing season. Another aspect to consider genetic factors, accounting for 60% of total factors. In adulthood decreases the neo-bone formation after a period in which bone mass remains stable (8).
Puberty brings the largest increase in bone mineral density in both sexes, however, as in any period may generate changes to diet and exercise as much as 20% (9). Bone mass is increased from birth to be reduced by calcium deposition significantly as we approach the third decade or so. To three years increases to 30% after 20% and reach puberty about 40%. From the end of growth and to reach adulthood is increasing by 15%. Even 10 years ago mineralization at the same rate in both sexes. From this age is accelerated significantly in the girls (10).
The diagnosis and even prevention evaluation and therapy of osteoporosis may be jeopardized in a special way in the child all because of the need to use techniques which, although sensitive, reproducible and precise, resulting quick, painless, safe and non invasive.
Of all the methods proposed by the National Osteoporosis Foundation to assess the quality of bone, the most used technique is dual x-ray absorptiometry (DEXA). The basis of this technique in the study of attenuation is subjected to a dual X-ray beam through bone tissue (11). Although it can be done at different levels, the benchmarks for determining the criteria of normality, osteopenia or osteoporosis referred to data obtained at the height of the femoral neck or lumbar vertebrae (L2-L4) of the reference population. The interpretation of this technique has some difficulties in the child (12). There are already benchmarks (13-15), although obtained in cross-sectional studies.
The measures are available in axial regions (hip, spine) or peripheral (calcaneus, tibia, knee, radio and phalanges), but it has shown that measurements in predicting spinal fracture risk at that level, but not others, and so does the rest of the locations where BMD is measured.

In children, the area selection is further complicated because the timing and rate of mineralization depends on the biological age (13). Should be selected sufficiently vascularized bone, with good motility and under some pressure. In this regard, the determination in the calcaneus could induce excessive bias to withstand pressure, although some authors is the preferred (14).
Other recent application techniques are ultrasound imaging and computed tomography. It is noninvasive, excellent acceptance of any age which have been effective as bone assessment procedures in both the adult and the child (15). However, in the case of computed tomography to excessive cost limits their use as a technique for the prevention of osteoporosis.

3. Bone mineralization process

Bone mineralization is a complex process regulated by both genetic and hormonal factors, environmental and nutritional (16). From a genetic standpoint, the mineralization is controlled by a large group of genes. Among the most studied is the gene that controls vitamin D receptor, which depends on calcium absorption in the intestine. Hormonal level, there are several hormones involved in bone mineralization. These include parathyroid hormone which balances the mechanisms of formation and resorption of bone at the same time enhances the action of vitamin D. Calcitonin, which inhibits the action of osteoclasts, and growth hormone, HGH and IGF-1 that acts in the formation of cartilage and promotes the synthesis of the active metabolite of vitamin D (17).
Other molecules with activity on bone mass are the corticosteroids. They only act on bone mineralization when increased above normal levels, decreasing bone mass and bone growth. This is an important consideration in those children treated with corticosteroids. Thyroid hormones, in turn, are also involved in mineralization diminishing with increasing concentration. But all of these factors may also act on the environmental factors that can intervene by modifying diet and lifestyle (18).

4. Concept of osteopenia and osteoporosis

Osteopenia is defined by decreased bone mineral density between -1 and -2.5 SD for age, sex, height and pubertal stage. In cases where the decrease in bone mineral density is below 2 SD is considered osteoporosis (19).
Osteoporosis was defined in 1991 as a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, which leads to increased bone fragility with a consequent increase in fracture risk. This definition implies a qualitative concept of altered bone architecture and a quantity related to bone density (20). Both osteoporosis and osteopenia may be primary as in aging or menopause but may also result from inadequate nutrition, and hormonal disorders or diseases of the bone.
However, there are childhood diseases that may present with osteopenia thereby increasing the risk of osteoporosis in adulthood. Among the mechanisms of production of osteopenia could cite many, though, could be divided into three main groups. Those processes that occur with an inadequate intake of nutrients such as anorexia nervosa, bulimia, protein-calorie malnutrition or poorly controlled diets (21). A second group would be composed of those disorders with intestinal malabsorption boxes. Within this section as possible symptoms of osteopenia generators could include celiac disease, cystic fibrosis, intolerance

to cow's milk proteins and inflammatory bowel disease. Other processes potentially involved in the development of osteopenia will neuropathy and liver disease that present with an impairment of the synthesis of active metabolites of vitamin D. Other processes involved will be the states of metabolic acidosis, prolonged administration of certain drugs such as anticonvulsants or corticosteroids and pictures of hypogonadism (22).

5. Nutritional factors

Proper nutrition is a key factor in maintaining adequate skeletal mineralization. In this process of bone mineralization energy and nutrients intervene in various ways, either by encouraging the development of cell mitosis, participating as visual elements, to be a source of vitamins which will involve regulating the synthesis of bone matrix and promoting the absorption level intestinal calcium or contributing to the synthesis of various hormones and factors crecimiento (23).

By feeding the body receives visual elements, vitamins intervene by regulating the synthesis of bone matrix and intestinal absorption of calcium and other minerals whose primary function is to act in the formation and consolidation of mineralized bone. Another essential aspect of bone remodeling in the child will be energy intake. This is an essential as the volume decreases in energy intake induce delays in growth, maturation and hence bone mineralization (24). Then in children with malnutrition by default is necessary to control the state of bone mineralization.

The bone mineralization process will necessarily regulated by protein intake through the diet. Its role essentially plastic makes these elements are essential for the synthesis of bone matrix. In this sense, the child, situations of inadequate intake may induce default to the emergence of problems of mineralization. On the contrary, when its contribution in the diet is excessive can cause hypercalciuria boxes, this is due to increased excretion of acid produced during protein catabolism. At present it is possible that the protein diet consumed in most developed countries it is closely linked with the increase in osteoporosis in the population (25).

Another aspect to consider is the ratio of sodium ingested with the level of calcium excretion by the kidney. Sodium and calcium share the same carrier at the proximal renal tubule. Although and yet there is no need to adjust the contribution of calcium to sodium intake through the diet in children (26). Calcium is an essential pillar in the prevention of osteoporosis. In our body and especially in the bones is deposited as hydroxyapatite crystals. Your deposit varies throughout life from 30 grams at birth to about 1.300 grams in adulthood (27).

Given the above will be necessary to modulate calcium intake during periods of increased growth and, especially during adolescence. During adolescence tends to accumulate 40% of total bone mass produced throughout life. Several studies have shown that calcium supplementation during adolescence increases bone mineral density (28). After administering 500 ml of milk per day during childhood will ensure intake of about 400mg of calcium, equivalent to 60% of the recommended daily amount.

Moreover, we have to take into account the bioavailability of calcium in food. The presence of phytates inhibit absorption and therefore vegetables, legumes and cereals despite containing high levels of calcium, it is not as comparable as that of milk. Similarly oxalates, alcohol, caffeine and phosphates hinder calcium absorption even when present in the diet (29, 30). Finally, pictures of obesity and overweight in children have been associated with

increased bone density. However there is evidence linking these situations with a higher incidence of fractures (31).
Vitamin D is another factor regulating the homeostasis calcium / phosphorus. Its main sources are dairy products. Exposure to sunlight or UV light promotes the metabolism of it. However, alterations in intestinal absorption mechanism and factors affecting their metabolism at the level of the skin should be considered as processes that alter bone formation and thus risk factors for developing osteoporosis (32).

6. Idiopathic juvenile osteoporosis

In some pediatric patients (usually young) are not able to establish any risk factors for osteoporosis. In these cases must be considered the possibility of presenting idiopathic juvenile osteoporosis. Its etiology is unknown, manifesting itself in some cases for an accidental radiological finding which may also require a significant osteopenia, short stature and kyphosis (secondary to vertebral crush fractures) (32). Generally do not exhibit any endocrine abnormality nor metabolism of calcium/ phosphorus. The levels of vitamin D and calcitonin are variable in these patients. For bone biopsy, this is not conclusive proof but often shows an increase of osteocytes in trabecular bone as well as signs of increased bone resorption. In general, treatment consists of substitution of calcium and calcitriol, tending to improve spontaneously in the post pubertal period by several authors due to the effect of gonadal hormones (33).

7. Osteogenesis imperfecta

Osteogenesis imperfecta is a genetic disease, autosomal dominant, in which there is an abnormality in the formation of collagen type 1 (34). This disorder causes weakness and bone fragility of varying degrees of severity and subsequent pathological fractures, as well as affecting other tissues. The etiology of this disease lies in the mutation of genes that encode both qualitative and quantitative production of collagen fibers. In terms of prevalence in the world, this ranges from about 1 case per 30,000 live births (34). The continuous advances in diagnosis have created new expectations for subjects with the disease, greatly improving their quality of life. At present there is no effective treatment, healing, since it can not act directly on the formation of collagen type I (34). Throughout history have used various medical treatments (calcitonin, anabolic steroids, etc.) to try to increase bone mass, to no avail. Currently, treatment is symptomatic and should be approached in a multidisciplinary manner. The best results were achieved with growth hormone (GH) and bisphosphonates (34).

8. Osteopathy associated with use of drugs

Another group of pediatric patients at high risk for osteoporosis are those subjects taking medications which interfere with the normal process of bone mineralization. The drugs most commonly associated with the development of bone disease or iatrogenic demineralizantes include steroids, anticonvulsants, cyclosporine, anthracyclines, methotrexate, warfarin and agonists of gonadotropin-releasing hormone (35).
In the case of steroids, these lead to the development of osteoporosis secondary to increased bone resorption. In addition, they inhibit intestinal absorption of calcium, decreased tubular

reabsorption of calcium and induce a secondary hyperparathyroidism. They also inhibit pituitary gonadotropin secretion and decrease the response of estrogen/testosterone to the follicle stimulating hormone (FSH). A level of osteoblasts caused a decrease in their ability to replicate, in turn stimulating the expression of collagenase by the osteoblast and thereby inducing the increase in bone matrix degradation with a decrease in the synthesis of growth factors (IGF1, IGF-2) (36).

Appropriate strategies to prevent osteoporosis from childhood: The prevention of osteoporosis to necessarily an assessment of bone mineralization status since early infancy, particularly in subjects at risk. In this sense, preterm infants, patients with malabsorption syndromes and corticosteroid therapy patients constitute the population most at risk of poor bone mineralization (37, 38).

The bioavailability of calcium in milk is far superior to commercial formulas, making it the leading source for calcium during breastfeeding. Only in the case of infants it should increase their calcium intake to the recommended supplementary with commercial formulas that have a higher calcium content (39).

In children aged 1 to 8 years there is no explicit consensus on the specific requirements of calcium. In any case, we recommend an intake of 500 mg per day for ages 1 and 3 years (40). This figure should be increased as they age and approach puberty. Thus, for ages 4 to 8 years the requirements will amount up to 800mg calcium per day. But have found no overt health benefits by increasing the daily amount (41). And at puberty, it is estimated that for every inch of growth are required calcio 20g (41).

Given the above, eating disorders, inflammatory bowel disease or the use of corticosteroids and prolonged rest in the minors are situations that require an attitude of monitoring and supervision by health staff (41).

It is estimated that the highest positive balance is achieved with an average daily intake of 1300mg. By contrast, those exposed to lower levels will have a negative impact on bone mineralization process (42). This corresponds to measurements made on white teens. In the case of blacks and adolescents have shown a better efficiency for the absorption of dietary calcium, can reach the same peak bone mass even with lower contributions of calcium (43).

Excess calcium in the diet, in turn, can cause a deficiency of iron and zinc, while favoring the formation of kidney stones (44). Similarly as phosphates present in carbonated drinks can also act by inhibiting the absorption of calcium in the intestine (45).

In cases of subjects with lactose intolerance, the simple addition of commercial lactase or ingestion of fermented dairy products like yogurt can remedy this situation (46).

The existence of toxic habits such as snuff or alcohol consumption can also interfere with the process of bone mineralization (47). But if there is a successful strategy to prevent osteoporosis from childhood this is the regular practice of exercise (48). The physical exercise from an early age not only ensure optimal weight status but also a formidable mineralization of our skeleton, reason is of great importance when the subject population are children and adolescents (49, 50). The continued practice of physical activity helps to acquire peak bone mass genetically determined (51). Although, to achieve these benefits, the current recommendations set out the need to practice a minimum of three days a week (52). Moreover, at present it is unknown whether calcium intake through diet may or may not alter the beneficial effect of exercise (52).

With regard to drug-induced osteoporosis, the most important preventive factor is the wise use and dosage of the same (53).

In summary, we conclude that the onset osteoporosis in children differs in its clinical management of osteoporosis in adults. In this sense, the early identification of risk grpos be a priority. Therefore it should be emphasized that during the first and second decade of life, events occur which are essential for the proper development of bone metabolism. On this basis, the prevention of osteoporosis in adults should begin as early as the early childhood.

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Physical Activity Interactions with Bone Accrual in Children and Adolescents

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1. Introduction

1.1 Osteoporosis and peak bone mass

Osteoporosis is a skeletal disease characterized by low bone mass and the deterioration of the micro architecture of bone tissue resulting in bone fragility and susceptibility to fractures (Gordon, 2003). According to the World Health Organization, osteoporosis is estimated to affect approximately 200 million women worldwide (Kanis, 2007) with the burden of osteoporosis being felt both personally and economically. Although the prevalence of fractures is higher is women, the mortality rate related to fragility fractures is higher in men (Center et al. 1999; Hasserius et al., 2003). Moreover, the annual cost of treating fractures in the United States is projected to increase to $25 billion in 2025 from $17 billion in 2005 (Burge et al., 2005).

Achieving peak bone mass (PBM) during adolescence and the subsequent rate of bone loss are major determinants of bone mass later in life (Hansen et al., 1991). The amount of bone mass achieved early in life has been shown to predict the level of bone mass and the incidence of fracture later in life suggesting that a primary risk factor for the development of osteoporosis is the inability to attain high PBM (Hansen et al., 1991; Heaney et al., 2000). PBM is generally defined as the highest level of bone mass achieved as a result of normal growth and seems to be established, for most sites of the skeleton, by late adolescence (Matkovic et al., 1994). Previous studies (Bonjour et al., 1991; Bailey et al., 1996) have demonstrated the period between 9-20 years of age to be critical in building peak bone mass as 90% of total body bone mineral content (BMC) is accrued by the age of 16 (Elgan et al., 2003; Stager et al., 2006), with the remaining 5-10% of total body bone mass achieved in the third decade (Cadogan et al, 1998). In fact, the most rapid bone mineral accumulation occurs approximately 1 year after the age of peak linear growth (Bailey et al., 1996); around the time of menarche for females (Cadogan et al., 1998). With considerable increases in bone mass occurring during puberty, maximizing PBM during this time is often advocated as the best way to delay age-related bone loss and prevent osteoporotic fractures (Fulkerson et al., 2004; Molgaard et al., 1999; Valimaki et al., 1994).

It appears, therefore, as though there is a critical period, a 'window of opportunity' (MacKelvie et al., 2002), in which we can influence the amount of bone mass we attain. However, bone development is the product of complex interactions between genetic and environmental factors including diet, hormonal influences, and mechanical stimuli (Gordon, 2003; Steelman & Zeitler, 2001). Permanent deficits in PBM are the result of any process that

interferes with normal bone mineral accretion during adolescence, such as inadequate calcium intake, physical inactivity, and poor lifestyle choices (related to smoking, alcohol consumption, carbonated beverages) (Javaid & Cooper, 2002). As a result, research in bone growth and development in youth has endeavoured to ascertain the factors important to increasing bone mineral accretion.

1.2 Physical activity

The use of physical activity (PA) in maintaining bone health throughout the lifespan and ultimately preventing osteoporosis has been the focus of considerable research in improving PBM in order to minimize later bone loss (Beck & Snow, 2003). It is generally accepted that engaging in PA during growth enhances bone development (Boot et al., 1997; Janz et al., 2001; Janz et al., 2006). Habitual PA has been shown to enhance lean mass (Baxter-Jones et al., 2008) and bone accrual (Baxter-Jones et al., 2003) in youth, both of which are believed to promote bone health and muscle function in older age (Lefevre et al., 1990). Furthermore, 'when' activity occurs during the lifespan is important as PA at a young age can account up to 17% of the variance in bone mineral density (BMD) seen in individuals in their late 20s (Davies et al., 2005).

In addition to the timing of PA, the method by which PA imparts its benefits on bone is also important. Mechanical loading of sufficient intensity to promote increases in skeletal mass during growth require maximal strains to be greater than those of normal everyday living. If the bone is properly overloaded the load will elicit a modeling response making the bone susceptible to new levels of mechanical demand (Bailey et al., 1996). Some of the largest loads placed on the skeleton are physiological ones resulting from muscle contractions (Rauch et al., 2004; Scheonau & Frost, 2002). Furthermore, gravitational or ground reaction forces are also capable of generating the loads necessary to elicit a favourable response in bone. These two loading methods have lead to investigations of bone responses to different forms of PA with comparisons between athletes and non-athletes. Studies have demonstrated athletes involved in high-impact weight-bearing activities such as gymnastics and running have higher BMD (Lehtonen-Veromaa et al., 2000b, 2000c) than athletes participating in low-impact sports such as swimming; with such athletes exhibiting lower or normal bone densities than non-active youth (Bellew & Gehrig, 2006; Cassell et al., 1996; Courteix et al., 1998). Resistance training and simple jumping exercises have also been shown to have positive effects on femoral BMD in adolescent females and as such can be useful in promoting bone growth and maintaining acquired gains (Fuchs & Snow, 2002; Kato et al., 2006; Nichols et al., 2001). Therefore, different forms of PA, such as resistance training (Nichols et al., 2001) and weight-bearing exercise (Fuchs & Snow, 2002; Lehtonen-Veromaa et al., 2000c) have been shown to have positive effects on the developing skeleton through ground reaction forces and muscle contraction.

Various studies have examined the relationship between PA and markers of bone metabolism (Creighton et al., 2001; Lehtonen-Veromaa et al., 2000a), with little research conducted on markers of bone formation and resorption in relation to different types of sports, particularly in children and adolescents. In female athletes between the ages of 18-26, Creighton et al. (2001) found bone formation to be lower and resorption similar in swimmers compared to basketball, volleyball, and soccer players. In a younger population of boys and girls, ages 9-16 years, no differences were found in any markers of bone metabolism between gymnasts (Lehtonen-Veromaa et al., 2000a), swimmers (Derman et al.,

2008) and controls. Therefore, research investigating the relationship regarding bone markers and different PA types is limited and ambiguous, but even more so in children and adolescents, making it difficult to ascertain the effect of sport on bone. The examination of biochemical measurements of bone turnover, in addition to static measures of bone, is advantageous in the study of skeletal metabolism and growth as they provide an understanding of the dynamic course of bone remodelling. To date, the use of biochemical marks of bone turnover in PA interventions on bone in youth has been extremely limited.

Difficulties in comparing and assessing the benefits of PA on bone during growth reflect the varying methodologies used between studies. PA interventions aimed at improving bone health in youth have been subject to limited maturational comparisons as the majority of interventions have been conducted in one distinct pubertal group. Furthermore, the types of PA interventions that have been applied have varied greatly between studies. Discrepancies in results are due in part to the varying bone assessment techniques that are used across cross-sectional and intervention studies. Many of the aforementioned studies measured improvements in BMD using dual-energy x-ray absorptiometry (DXA). The use of DXA to interpret and evaluate BMD in the growing years can be difficult as there are considerable changes to the size and shape of bone (Bailey et al., 1996; Gordon, 2003; Schoenau et al., 2004), making comparisons between youth problematic. Furthermore, the measurements provided by DXA fail to account for the architecture, organization of tissues, mechanical properties and other factors known to impart bone strength. In addition, the bone assessment techniques used in majority of these studies have provided a static rather than dynamic picture of bone, which could in fact allow for more comparisons across studies.

Evidence supporting the role of PA on bone health has been accumulated from a wide range of studies investigating different activity methods using athletes, non-athletes and inactive individuals. Although these studies contribute to the literature they do not provide us with causality that PA does impart benefits to bone health. In response, there has been an increase in the number of intervention studies conducted, particularly in the school setting. PA interventions in schools are in many ways ideal places to intervene as they allow for a large population of children and adolescents to be targeted in a somewhat controlled environment, regardless of socioeconomic status, in a location where youth already spend a majority of their day during their most skeletally responsive years (Hughes et al. 2007).

2. Methods

Therefore, the primary objective of this chapter is to conduct a systematic review on the effectiveness of exercise and PA interventions to improve bone accrual in children and adolescents. Key finding from controlled intervention trials using various techniques to assess bone mineral density, content and strength changes will be discussed and be grouped according to maturity status. This will hopefully help to shed light on the best time during growth and development to influence bone health and to ascertain if there is indeed a window of opportunity for bone response.

We will also discuss and compare the different types of interventions used to affect changes in bone properties in youth, to determine if there is a modality that is best suited to improving bone development and to what degree these interventions influence changes in bone. Furthermore, we will address the characteristics of loading that have been shown to be best associated with particular structural improvements as interventions can be designed to impart mechanical loading on bone by jumping or by resistance training

where the weight-bearing load on bone is applied through muscle. As majority of interventions measure only static properties of bone, this chapter will also be used to discuss bone remodelling parameters influenced by such exercise interventions. To our knowledge there has not been any studies examining the effects of PA interventions on bone remodelling.

2.1 Eligibility criteria and search strategy

The aim of the literature search was to find all available randomized control trials and controlled studies that examined the effects of any type of exercise or PA intervention trial on bone status in healthy (non-clinical, non-athletes) children and adolescents between 6 and 17 years of age. For this review we included all types of bone parameters from various bone assessment techniques (DXA, pQCT, QUS etc.) to be used as primary outcome measures as long as there were at least two measurement time points. Primary outcome measures included areal bone mineral density (aBMD), volumetric bone mineral density (vBMD), bone mineral content (BMC), bone area (BA), cortical thickness, bone strength index (BSI), stress-strain index (SSI), maximal moment of inertia (I^{mas}), section modulus (SM), speed of sound (SOS), broadband ultrasound attenuation (BUA), and markers of bone metabolism.

A computerised search of the MEDLINE and PubMed databases was performed on articles up till 2011 using a comprehensive combination of keywords to describe exercise, bone and participant parameters. The keywords used to describe exercise included: intervention and intervention studies, training, exercise, resistance training, physical education and physical education training, physical activity and motor activity. Bone parameter keywords included: bone mineral, bone density, bone and bones, bone strength, bone accrual and development, bone turnover, resorption, modelling and metabolism. For the participants, keywords such as children, adolescents, boys and girls were used. A total of 2728 were found, their titles and abstracts reviewed to determine if they met the inclusion criteria. Papers from all journals were considered and retrieved electronically or by interlibrary loan.

After screening the articles a total of 35 studies met the criteria and were used for the current review. Studies were grouped according to the maturity status of their participants based on Tanner Staging of development (Tanner, 1962). Participants were grouped as either prepubertal (Tanner 1), early pubertal (Tanner 2 and 3), and pubertal (Tanner 4 and 5) to maintain consistency with other literature review groupings. Studies in which authors provided results for more than one maturity group were divided into two parts (A and B).

3. Results

Table 1 represents the numerical breakdown of all the intervention studies reviewed into particular categories based on the type of intervention that was used, the method in which bone parameters were assessed, the maturity and sex of the population measured. Studies were included more than once if more than one measurement technique was used and if results were separated by sex or maturity group. Table 2 is a detailed summary of the design and outcomes of all the PA intervention studies reviewed, and are grouped according to the participants' maturity status. The results presented in Table 2 express the percentage difference in gain between the experimental groups participating in the intervention in

comparison to controls. The results presented in the Table 2 are the final finding after any statistical adjustments have been made.

Type of Intervention		Measurement Technique		Maturational Status		Gender	
School Based		SXA	1	Prepubertal	16	Boys	12
Part of PE Class	23	DPA	1	Early Pubertal	16	Girls	24
At the School	5	DXA	33	Pubertal	7	Boys + Girls	7
Outside School	7	HSA	4	Multi Pubertal^{separate}	4		
Jumping	18	pQCT	5	Multi Pubertal^{together}	5		
General WBPA	14	QUS	3				
Resistance Training	3	Bone Markers	1				

Table 1. Numerical Breakdown by Category of Exercise Interventions for Bone in Youth

Prepubertal corresponds to Tanner Stage 1, early pubertal Tanner Stages 2-3, and pubertal Tanner Stages 4-5. Multi pubertal *separate* are studies with results separated by maturity, with *together* being studies that averaged data for more than one maturity group. Boys + girls reflect studies that did not separate results by sex. PE: physical education; WBPA: weight-bearing physical activity; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound.

Majority of the intervention studies were school based with 23 of the studies being conducted as part of a regular physical education class and 5 at some point within the school day. Approximately half (51%) of the studies utilized specific jumping interventions that relied on ground reaction forces in order to elicit a positive response on bone. Fourteen studies consisted of general weight bearing types of activities such as running, volleyball, aerobics etc., with only 3 studies specifically using resistance training with free or machine assisted weights. Significant increases in primary bone outcomes were found in 16 jumping interventions, 14 WBPA interventions, and 1 resistance training study. This translated into 79.5% of physical activity interventions positively influencing some form of bone strength parameter in children and adolescents. Furthermore, 5 studies also included calcium interventions which demonstrated benefits to bone in addition to physical activity.

Of the 35 studies reviewed 24 presented results separately for girls, 12 for boys, with 7 studies presenting data for boys and girls together. Moreover, 16 studies conducted interventions in prepubertal and early pubertal children. The smallest number of studies was performed in pubertal youth with a total of 7. All the pubertal interventions were completed on a population of girls, with 1 study (Weeks et al., 2008) including boys in their sample. Based on pubertal groups, an even number of boys and girls were represented in the results of prepubertal youth with 8 studies separately reporting results for boy and girls and 2 grouping results together. In early pubertal children, a larger number of studies were conducted on and included girls. Ten studies reported results separately for girls, 3 for boys and 5 did not distinguish results between genders.

DXA was the measurement technique predominantly used (94%) to assess bone, followed by pQCT (14%) and then QUS (8.5%). In total, 5 studies used more than one technique to determine changes in bone and these were all done in conjunction with DXA measurements. Four studies using DXA also performed hip structural analysis (HSA), which is a new

application for DXA allowing for the estimation of geometric contributions to bone strength in the proximal femur and may potentially provide a better representation of bone strength (Bonnick, 2007). It is surprising that such a large percentage of studies utilized DXA given the known methodological issues with assessing changes in bone during growth. Until recently, we had thought no intervention studies had used biochemical markers of bone metabolism. Our extensive literature search found 1 study (Schneider et al., 2007) that measured serum markers of bone formation and resorption in adolescents. As static measures require longer durations for differences to be found, measuring biochemical markers of bone turnover to assess dynamic properties of bone could be advantageous in detecting changes sooner and allow for better comparisons of results between studies.

3.1 Prepubertal interventions

Positive effects of exercise on bone indices were found in 13 of 16 studies (81%), with overall effects ranging from 0.6% to 9.5% depending on the skeletal location and the type of measure (BMC, BMD, etc) taken for studies 7-36 months in duration. The average percent improvements for BMC included 4.5%, 4%, 2%and 1.5% at the lumbar spine (LS), femoral neck (FN), femur and total body (TB) respectively. BMD gains across studies were between 0.6-3% for the LS, FN and TB. The largest gains in girls was in BMC and area of the forearm (12.5% and 13.2% respectively) using peripheral DXA after 36 months of increased physical education class time (Hasselstrom et al., 2008). The one study that used pQCT in this group (Macdonald et al., 2007) was also the study that exhibited the largest bone gains in boys after 16 months of jump training, finding an increase of approximately 25% in BSI (an index of bone structural strength) of the distal tibia. MacKelvie et al. (2004) also presented large gains using HSA, with boys seeing a 12% increase in FN cross-sectional moment of inertia.

Despite the bone gains being similar between boys and girls, the number of studies that reported significant findings differed (4 vs. 7 out of 8 for girls vs. boys respectively). These discrepancies can largely be explained by the differences in the length and type PA intervention employed. MacKelvie et al. (2001) and (2002) were studies that utilized 7 months of school based physical education classes to employ a jump circuit intervention eliciting ground reaction forces 3-5 times one's body weight and demonstrated favourable gains in bone in boys but not girls. Fuchs et al. (2001) also found 7 months of jump training to be favourable to improvements in LS and FN BMC and BMD in prepubertal boys and girls. In fact the gains demonstrated in Fuchs et al. (2001) were greater than those in the MacKelvie et al. (2001, 2001) studies, most likely due to the larger ground reaction forces generated (8.8 vs. 3.5-5 x body weight). Studies at 12 months (Alwis et al., 2008b; Linden et al., 2007) utilizing a weight bearing physical education intervention follow a similar trend with improvements being seen in boys but not girls. The extra intervention time has not helped to elicit a significant positive bone response in the young girls. It is not till 24 months of the same type of weight bearing PA intervention that positive gains are found in girls (Linden et al., 2006). It would therefore appear that improvements in bone as a result of a PA intervention would more likely occur in prepubertal boys than girls. This is particularly true after 7 months of jumping training (MacKelvie et al., 2001, 2002) and 12 months of weight bearing PA (Alwis et al., 2008b; Linden et al., 2007). Improvements in prepubertal girls were seen in studies lasting 24 months in duration (Linden et al. 2006) and any studies demonstrating bone gains in a mixed gendered population (Fuchs et al., 2001; McKay et al., 2000) could be due to greater changes in the boys than the girls.

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
Alwis et al. (2008a)	Boys, White Ex: n=80, Con: n=57 Age range: 6.7-9 yrs All remained TS 1 Randomized by school: 1 Ex + 3 Con.	24 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Compliance: Con 84%, Ex 95%	DXA BMC: total body and L3 vertebra L3 vertebral width HSA of femoral neck	BMC L3: +3% L3 width: +1.3%	Uneven sample size between Ex and Con. Accelerometers captured only 4 days of 2-yr intervention Compliance not reported
Alwis et al. (2008b)	Girls, White Ex: n=53, Con: n=50 Age range: 6.7-9 yrs All remained TS 1 Randomized by school: 1Ex + 3 Con.	12 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Compliance: Con 76%, Ex 95%	DXA and HSA BMC, aBMD, periosteal and endosteal diameter, cortical thickness, CSMI section modulus, and CSA of FN	No significant between group differences were found	Follow up periods varied Higher spare time activities in control group.
Bass et al. (2007)	Boys, White + Asian Total n=88, 7-11 yrs Ex Placebo: n=21 Ex Ca: n=20 No Ex Ca: n=21 No Ex Placebo: n=26 Randomized groups Ca: double blind	8.5 Months Part of PE class: 20min 3x week Hopping jumping, skipping moderate or low impact Ex: Ground rx forces 2-8 x BW No Ex: Ground rx forces 1 x BW Ca: 800mg Ca/day Compliance 86%	DXA BMC: total body, lumbar spine, femur, tibia-fibula, humerus, radius-ulna	Femur BMC: +2% Ex+Ca > all other grps Tibia-fibula BMC: +2% ExCa>Ex Placebo +3% Ex Ca> No ExCa and No Ex Pl NS for BMC in arms	Low sample sizes in each of the groups Control grp participated in low impact exercise making possible differences between groups smaller Population not all TS1 61% TS 1, 39% TS 2
Bradney et al. (1998)	Boys, White N=20 Ex, m=20 Con Age range: 8.4-11.8 All remained TS 1 Randomized by school: 1 Ex + 1 Con	8 Months Program outside of school: aerobics, soccer, volleyball, dance, gymnastics, basketball, weight training 30 minutes, 3 x week	DXA aBMD: total body and lumbar spine, femur, Femoral Midshaft BMC, aBMD and vBMD, and cortical thickness	aBMD TB: +1.2% aBMD LS: +2.8% BMC and aBMD femoral midshaft: +5.6% cortical thickness: +6.4%	Low sample sizes in each of the groups volumetric bone densities were derived/estimated
Fuchs et al. (2001)	Boys and Girls, Asian and White Age range: 5.9-9.8 yrs n=45 Ex., n=41 Con Randomized 1 school All remained TS 1	7 Months Activities added to PE classes: 10 min 3x week jumping 50-100 high box jumps, 2 footed Ground rx forces = 8.8 x BW 90% Compliance	DXA BMC and aBMD: lumbar spine and femoral neck BA: femoral neck	BMC LS: +3% BMC FN: +4.5% aBMD LS: +2% aBMD FN: NS BA FN: +2.9%	cannot distinguish results between boys and girls

Table 2. Randomized and Non-Randomized Controlled Studies on the Effects of Exercise on Bone Indices in Youth

Table 2. Continued – Studies on the Effects of Exercise on Bone in Youth

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
Hassel-strom et al. (2008)	Boys and Girls, White (Ex: n= 135 and 108) (Con: n= 62 and 76) Age Range: 6-8 No Randomization TS 1 and 2	36 Months School based curriculum, time increased: 4 classes 180 min/wk Con: regular school curriculum 90min/wk Activities conducted in classes not mentioned	Peripheral DXA BMC and BMD: Calcaneus and distal forearm	Girls: NS changes in calcaneal and distal forearm BMD BMC forearm: +12.5% forearm area: +13.2% Boys: NS changes in all measures	Non-randomized study design allowing for selection bias DXA locations measured less studied Possible differences in standard anatomical region measured due to growth
Linden et al. (2006)	Girls, White Ex: n=49, Con: n=50 Age range: 7-9 All remained TS 1 Randomized by school: 1 Ex + 3 Con.	24 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Ex. Attendance: 90%	DXA BMC and aBMD: TB, LS L2-L4 and L3, FN, and Leg vBMD, bone size: L3 and FN	BMC: L2-L4 +3.8%, L3 +7.2%, Leg +3.0% aBMD: TB +0.6%, L2-L4 +1.2%, L3 +1.6%, Leg +1.2% Bone Size: L3 +1.8%, and FN +0.3%	Differences in leisure time PA Compliance not reported
Linden et al. (2007)	Boys, White Ex: n=81, Con: n=57 Age range: 7-9 All remained TS 1 Randomized by school: 1 Ex + 3 Con.	12 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk Ex. Attendance: 90%	DXA BMC and aBMD: TB, L3 vertebra, FN Bone Width: L3 and FN	BMC, aBMD, bone width L3: +5.9%, +2.1% and +2.3%	Uneven sample size between Ex and Con. Compliance in Con Low Only assessed duration of PA, not intensity or effort
Macdonald et al. (2007) (Part A)	Boys and Girls Asian and White Ex: n=140, Con: n=72 Age range: 9.6-10.8 Randomized by school: 7 Ex. + 3 Con.	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	pQCT BSI distal tibia SSI tibial midshaft	Boys: BSI distal tibia increased ~+25% Girls: NS changes in all measures	Low Compliance Potential bias for school selection Low Compliance Uneven sample sizes and distribution of sexes
MacKelvie et al. (2001) (Part A)	Girls, White + Asian Ex: n=44, Con: n=26 Age range: 9.4-10.6 Randomized by schools: 7 Ex + 7 Con	7 Months Activity added to regular PE class: 10min, 3 x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 80% across schools	DXA BMC and aBMD: TB, LS, PF, FN vBMD: FN	NS differences in any of the bone variables measured	vBMD measurements were derived/estimated Uneven sample size between Ex and Con. More Ex's advances from TS 1 to TS2

Table 2. Continued – Studies on the Effects of Exercise on Bone in Youth

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
MacKelvie et al. (2002)	Boys White + Asian Ex: n=61, Con: n=60 Age range: 9.7-10.9 Randomized by schools: 7 Ex + 7 Con	7 Months Activity added to regular PE class: 10min, 3 x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 80% across schools	DXA BMC and aBMD: TB, LS, PF, FN vBMD: FN	BMC TB: +1.6% aBMD PF: +1%	vBMD measurements were derived/estimated
MacKelvie et al. (2004)	Boys, White + Asian Ex: n=31, Con: n= 33 Age range: 9.6-10.7 Randomized by schools: 7 Ex + 7 Con	20 Months Activity added to regular PE class: 10min, 3 x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW	DXA and HSA BMC and BA: TB, LB, PF, FN, and TR HAS: PF, NN, TR , FN SM: FN	BMC FN: +4.3% Cross-sectional moment of inertia: +12.35% SM: +7.4%	Study compliance: Ex 39% and Con 42% More Con remained TS 1 and more Ex's advanced to TS 3
McKay et al. (2000)	Boys and Girls White and Asian Ex: n=63, C: n=81 Age range: 6.9-10.2 School randomized	8 Months Part of PE classes: jumping, hopping, skipping 2 x week 3 x week 10 tuck jumps Con: regular PE classes	DXA aBMD: TB, LS, PF, FN, and trochanter (TR)	aBMD TR: +1.2%	All boys remained TS 1, with some girls maturing to TS 2 Compliance not reported
Petit et al. (2002) (Part a)	Girls, Asian + White Age range: 9.4-10.6 Ex: n=43, Con: n=25 Randomized by schools: 14 schools ethnic stratification	7 Months Part of PE classes: 10-12 min 3x week: 5 x diverse jumping exercise stations Con: regular PE classes Ground rx forces=3.5-5 x BW	DXA and HSA abed: TB, LS, TR, PF cortical thickness, area and SM: PF	NS differences in any of the bone variables measured	Compliance not reported Errors related to method of measurement
Valdimar-sson et al. (2006)	Girls, White Ex: n=53, Con: n=50 Age range: 7-9 yrs Ex group come from one school	12 Months Typical PE class: ball games, running jumping, climbing Ex: 40min/day (200min/wk) Con: 60min/wk. 90% Attendance	DXA BMC and aBMD: TB, LS (L2-L4), L3, FN, leg vBMD: L3 and FN	BMC LS: +4.7% BMC L3: +9.5% aBMD LS: 2.8% aBMD L3: 3.1% Bone width L3: +2.9%	No randomization Compliance low in controls volumetric bone densities were derived/estimated

Table 2. Continued – Studies on the Effects of Exercise on Bone in Youth

Reference	Population	Intervention	Measures	Results	Limitations
Pre Pubertal (Tanner Stage 1)					
Van Lang-endonck et al. (2003)	Girls Ethnicity not reported Ex: n=21, Con: n=21 21 pairs of monozy-gotic twins Age range: 8-9yrs	9 Months Ex: 3x week: hopping/jumping Progression: removal of shoes different stimulus Ground rx forces not measured Compliance: Ex 91%	DXA BMC, aBMD, BA: FN and PF	BMC PF: +2.5% aBMD PF: +1.3% BMC FN: +2.0% aBMD FN: +2.4%	Some of the girls participated in high impact sports during their leisure time - separate analysis conducted
Early Pubertal (Tanner Stage 2-3)					
Barbeau et al. (2007)	Girls, Black n=77 Ex., n=83 Con. Age range: 8-12 yrs Recruited from 8 elementary schools	10 Months After school intervention 5 days/week, 80 min PA: 25min skills, 35min MVPA, 20min toning + stretching	DXA Total body BMD, BMC	BMC TB: +4.0% BMD TB: +2%	Examined girls who attended 40% of classes 2d/wk Main focus was to improve cardiovascular fitness Low compliance
Courteix et al. (2005)	Girls, White (n=85) Age range: 8-13 yrs Ex Ca: n=12 Ex Placebo: n=42 No Ex Ca: n=10 No Ex Placebo: n=21 Randomized, Blinded	12 Months Ex: 7.2h/week No Ex: 1.2h/week Ca: 800 mg/day Compliance 75% Ex: Participated in weight bearing physical activity	DXA aBMD: TB, LS, FN, WT	aBMD TB: +6.3% aBMD LS: +11% aBMD FN: +8.2% aBMD WT: 9.3% (all Ex Ca > No Ex Pl) NS differences between other groups	Uneven sample size distribution between groups Type of exercise not controlled Exercise based on habitual activity
Heinonen et al. (2000) (Part A)	Girls, White Ex: n=25, Con:, n=33 Age range: 10-12yrs Selection to groups decided by teachers	9 Months Step aerobic program: 50 min 2 x week: 20 min of jumping exercises: 100-200 jumps from box (two and one footed) Ground rx forces not measured Compliance: Ex 73%, Study 92%	DXA and pQCT BMC: LS, FN, and TR Cortical area: tibial midshaft	BMC LS: +3.3% BMC FN: +4.0%	Compliance low Potential selection bias due to teachers selecting groups
Iuliano-Burns et al. (2003)	Girls, White + Asian Total n=64 Age range: 8-9 yrs Mod Ex. Ca: n=16 Mod Ex. Pl: n=16 Low Ex. Ca: n=16 Low Ex. Pl: n=16 Randomized groups	8.5 Months Ex: 20 min 3 x week Mod Ex. Impact: skipping, hopping, jumping. Used hand weights in final 8 weeks Low Ex. Impact: stretching Ca: average of 434 mg/day Compliance: Ex 93%, Study 88%	DXA BMC: LS, Femur, Tibia-Fibula	BMC tibia-fibula: +3% Mod ex>Low Ex. +7.1% Mod Ex Ca > Low Ex. No Pl.	Low sample sizes

Table 2. Continued - Studies on the Effects of Exercise on Bone in Youth

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
MacKelvie et al. (2001) (Part B)	Girls, White + Asian Ex: n=43, Con: n=64 Age range: 9.9-11.1 yr Randomized by schools: 7 Ex + 7 Con	7 Months Part of PE class: 10min 3x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 80% across schools	DXA BMC and aBMD: TB, LS, PF, FN Volumetric BMD: FN	BMC LS +1.8% aBMD LS +1.7% BMC FN: NS aBMD FN: +1.6% vBMD FN: +3.1%	Volumetric bone densities were derived/estimated Uneven sample size between Ex and Con.
MacKelvie et al. (2003)	Girls, Asian + White Ex: n=33, C: n=43 Age range: 9.3-10.7 Randomized by schools: 7 Ex + 7 Con	20 Months Part of PE class: 10min 3x week 50-100 jumps and circuit training, progressing w/jumps Jumping = 3.5-5 x BW Compliance 42% over 20 Mos.	DX BMC: LS and FN	BMC LS: +3.7% BMC FN: +4.6%	Con group older and more mature Compliance not reported for Ex. Group
Macdonald et al. (2007) (Part B)	Boys and Girls Asian and White Ex: n=135, Con: n=57 Age range: 9.6-10.8 yrs Randomized by school: 7 Ex. + 3 Con.	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	pQCT BSI distal tibia SSI tibial midshaft	NS changes in any of the measures	Low Compliance Potential bias for school selection Uneven sample sizes and distribution of sexes between groups
Macdonald et al. (2008)	Boys and Girls Asian and White Ex: n=140, Con: n=72 Age range: 9-11 yrs Randomized by school: 7 Ex. + 3 Con. TS 1-3	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	DXA and HSA FN bone strength, geometry, and BMC BMC: TB, PF, LS	Boys: BMC LS: +2.7% BMC TB: +1.7% Girls: section modulus of FN: +5.4% (only in girls with 80% compliance)	Low teacher compliance Uneven sample sizes and distribution of sexes btw grps More boys prepubertal and girls early pubertal Results not separated by maturity status
Macdonald et al. (2009)	Boys, Asian + White Ex: m=139, Con: n=63 Age range: 9-11 yrs Randomized by school: 7 Ex. + 3 Con.	16 Months Ex: 15 min/day PA 5 x week, 5-36 jumps/day 4 x week Con: regular school curriculum Compliance 74%	pQCT Second moments of area, cortical area, cortical thickness of tibia	Max second moment of area: +3% Trends for increase in cortical area and thickness, but NS	Uneven sample sizes Higher percentage of TS2 in Ex Group compared to Con at baseline, with Con having more TS1

Table 2. Continued - Studies on the Effects of Exercise on Bone in Youth

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
McKay et al. (2005)	Girls and Boys Asian and White Ex: n=51, Con: n=73 Age Range: 9.5-10.5 No Randomization	8 Months Program: Bounce at the Bell 10 counter movement jumps 3 min 3 x day each school day Ground Rx forces: 5 x BW Compliance: Ex 60%, study 100%	DXA and HSA BMC: PF and TR BA: PF and TR Cortical thickness and area: PF	BMC PF: +2.0% BMC TR: +2.7% BA PF: +1.3% BA TR: +2.0% Con > Ex: BMC and BA TB	Compliance Low Ex group participated in greater PA at baseline Con greater increase in TB BMC and BA
Meyer et al. (2011)	Boys and Girls, White Ex: n=297, Con: n=205 Age range: 6.6-11.7 yrs Randomized by classes: Ex: 16 classes/ 9 schools, Con: 12 classes/6 schools TS 1-3	12 Mos School based program Ex: regular PE class + 2 extra PE classes that include 10 min jumping activities. 2-5min jumping/balancing tasks through out day Con: regular PE classes	DXA BMC and aBMD: TB, FN, L2-L4	BMC TB: +5.5% BMC FN: +5.4% BMC LS: +4.7% aBMD TB: +8.4% aBMD LS:+7.3% Pubertal stage*group interaction favored prepubertal children	Has distinct pubertal groups but results not separated by maturity. Maturity used to adjust for variables Small sample size of pre pubertal Con grp (loss of data) Compliance not reported
Morris et al. (1997)	Girls, Ethnicity not given, but schools stratified according to ethnicity Ex: n=38, Con: n=33 Age range: 8.6-10.4 yrs No randomization Grouped by teachers	10 Months Activity added to regular PE class: 30 min 3 x week Aerobics, skipping, dance, ball games, progressing to weight training Ground rx forces not measured Compliance: Ex 92%, Study 97%	DXA and BMAD BMC: TB, LS, FN, PF aBMD: TB, LS, PF BMAD: LS, FN	BMC TB and LS: +5.5% BMC FN: +4.5% BMC PF: +8.3% aBMD TB: +2.3% aBMD LS: +3.6% aBMD FN: +10.3% aBMD pF: +3.2% BMAD LS: +2.9%	Potential selection bias as teachers selected groups Maturity greater in Ex than control (due to drop outs) and could contribute to the greater gains seen
Nemet et al. (2006)	Boys and Girls, Ethnicity not given Ex: n=12, Con: n=12 Age range: 6-16 yrs Obese participants Randomized groups	3 Months Structured activities to mimic PE classes. Mainly endurance: 50% sports, 50% running and games: 1 hour 2 x week Received nutrition counseling	QUS SOS of left tibia	SOS: +2.9% Difference due to significant SOS decrease (-2.6%) in Con, and NS increase in Ex. (+0.6%)	Small sample size Population spans a large age range Compliance not reported

Table 2. Continued - Studies on the Effects of Exercise on Bone in Youth

Reference	Population	Intervention	Measures	Results	Limitations
Early Pubertal (Tanner Stage 2-3)					
Nichols et al. (2008)	Boys and Girls, White Total n=112 Age range: 9-10yrs Ex only: n=61 Nutrition only: n=9 Ex + nutrition: n=14 Con: n=28 4 schools randomized 85% TS1 at baseline	20 Months Activity added to PE classes: 8-12min 2 x week: of jumping and skipping Ground Rx forces 2-3 x BW Nutrition: 45min biweely clasees to improve Ca intake Compliance: 80% at 8 months, 73% at 20 months	DXA BMD: TB, LS (L2-L4), PF, and FN BMC: TB, LS, FN, PF Measures taken twice: 8 and 20 months	NS differences between groups for any of the bone measurements taken at 8 and 20 months	Uneven sample size distribution between groups TS estimated based on height velocity Leisure PA not controlled: 59% reported participating in organized sports/activities Ground rx forces estimated
Petit et al. (2002) (Part B)	Girls, Asian + White Age range: 9.9-11.1yrs Ex: n=43, Con: n=63 Randomized by schools: 14 schools stratified by ethnic composition	7 Months 10-12 min 3x week 5 x diverse jumping exercise stations Activities done in addition to regular PE classes Con: regular PE classes Ground rx forces=3.5-5 x BW	DXA and HSA aBMD: TR and FN SM: FN cortical thickness: FN	aBMD TR: +1.7% aBMD FN: +2.6% SM FN: +4.0% cortical thickness FN: : +3.2%	Compliance not reported Errors related to method of measurement
Sundberg et al. (2001)	Boys and Girls, White Ex Boys: n=40 Ex Girls: n=40 Con Boys: n=82 Con Girls: n=66 Age range: 12-16 yrs 2 Schools (1 Ex, 1 Con) Recruited grade 6,7 (12-13yrs), follow up grade 9 (15-16yrs) TS 2,3 start TS 4,5 end	3-4 Years Additional time in PE classes Ex: 40min 4 x week 3 of 4 classes: weight bearing activities, jumping, running, gymnastics, ball games 1 of 4 classes: swimming Con: regular PE classes of 60 min 2 x week Compliance: Ex 93%, Con 91%	DXA: BMC, aBMD, vBMD, and bone size: TB, LS, FN SXA: BMC and aBMD: distal radius and ulna ultradistal radius QUS: BUA, SOS, and SI: calcaneus (heel)	3/4 Years Boys: BMC FN: +8% / 0% aBMD FN: +9% / +14% vBMD FN: 9% / +15% BMC LS: +9% / 0% aBMD LS: 0% / +10% SOS Heel: +1% / +11% SI Heel: +7% / +2% 3-4 Years Girls: aBMD distal/ultra-distal radius: -6-7%	vBMD and BA was derived Con girls had high levels of leisure PA, bone mass, Ca intake and earlier menarche than Ex girls, which may have masked effects of intervention Ex program not specific to building bone Control group not from the same school
Pubertal (Tanner Stage 4-5)					
Blimkie et al. (1996)	Girls Ethnicity not reported Ex: n=16, Con: n=16 Age range: 15.9-16.3 All postmenarcheal	6.5 Months Machine assisted weight training 3 x week 4 sets of 12 reps each, with progression every 6 weeks	DPA BMC: TB and LS aBMD: TB and LS	NS differences in any of the bone variables measured	Compliance was no clear The duration/length of each session was not clear

Table 2. Continued - Studies on the Effects of Exercise on Bone in Youth

Reference	Population	Intervention	Measures	Results	Limitations
Pubertal (Tanner Stage 4-5)					
Heinonen et al. (2000) (Part B)	Girls, White Ex: n=39, Con:, n=29 Age range: 12.8-15yrs Selection to groups decided by teachers	9 Months Step aerobic program: 50 min 2 x week with 20 min of jump exercises: 100-200 jumps from box (two and one footed) Ground rx forces not measured Compliance: Ex 65%, Study 92%	DXA and pQCT BMC: LS and FN Cortical area: tibial midshaft	NS differences in any of the bone variables measured	Compliance low Potential selection bias due to teachers selecting groups
Nichols et al. (2001)	Girls Ethnicity not reported Ex: n=5, Con: 11 Age range: 14-17 yrs All postmenarcheal Randomized groups	15 Months Resistance training program weights and machines: 30-45 min, 3 x week of 15 Progression: weight increase Compliance: Ex. 73%, Study 15%	DXA BMC and aBMD: TB, LS, FN, WT, and TR BMAD: LS and FN	aBMD WT: +3.2% aBMD FN: +2.3%	Large dropout rate resulting in small sample size (originally Ex=46, Con=21)
Schneider et al. (2007)	Girls, White, Hispanic, Asian Ex: n=63, Con: n=59 Age range: Randomized two schools: 1 Ex + 1 Con All given 500mg Ca/d	10 Months, 2 school semesters School based program: 60 min 5 x week (~40min activity time) Variety of aerobic (3 x week), strength building (1 x week), educational (1 x week) activities	DXA + bone turnover BMC and BMD: TB, LS, Hip, thoracic spine, FN and TR Bone formation: OC, BSAP, and CICP Bone resorption: PYD	Thoracic BMC: +4.9% NS differences in BMD measurements or markers of bone turnover	Compliance not reported Population may not be generalizable as proactive approach to attrition taken and terminated participation Duration of study time points is unclear
Stear et al. (2003)	Girls, White Total n=144 Age range: 16-18 yrs Ca Ex: n=37 Ca No Ex: n=28 Placebo Ex: n=38 Placebo No Ex: n=28 All postmenarcheal Randomized, double blinded 2 schools	15.5 Months Lunch + after school program 45min 3 x week of aerobic to music: moderate to vigorous high impact movements Ground rx forces not measured Ca: 1000mg/day Ex attendance: 36% Ca compliance: 70%	DXA BMC and BA: TB, LS, FN TR, hip, nondominant total, ultradistal and distal third radius	Ca Ex > Placebo No Ex BMC TB: +0.8% BMC LS: +1.9% BMC FN: +2.2% BMC Hip: +2.7% BMC TR: +4.8% Ex > No Ex BA LS: +0.7% BMC Hip: +1.4% BMC TR: +2.6%	Poor Ex attendance Decreased BA in the hip which may suggest reorientation of the hip with increasing age, redistribution of mineral, or alternation in bone-edge detection of DXA Results based on good compliance (smaller sample)

Reference	Population	Intervention	Measures	Results	Limitations
Pubertal (Tanner Stage 4-5)					
Weeks et al. (2008)	Boys and Girls Total n=81 Ex Boys: n=22 Con Boys: n=15 Ex Girls: n=21 Ex Girls: n=23 Age range: 13.5-14.5 Randomized 1 school	8 Months Ex: 10 min 2x week jumping activities as warmup in PE class worked up to ~300 jumps at 1-3 Hz, height 0.2-0.4m Con: 10min 2x week of regular PE class warmup Compliance Ex 80% Study dropout rate 18%	DXA and QUS BMC, BMD, and BA: TB, FN, LS, TR BMAD, CSMI, IBS, and cortical wall thickness BUA: nondominant calcaneus	Boys: BMC TB: +4.3% NS increases Ex boys: BUA calcaneus: +3.6% FN area: +1.1% Girls: NS differences NS increases Ex girls: BMC FN: +9% BMAD LS: +3.7% LS area: +2.9%	Volumetric bone densities were derived/estimated Small sample size for between group sex differences
Witzke & Snow. (2002)	Girls, White Ex: n=25, Con:, n=28 Age range: 14-15 yrs All postmenarcheal No randomization	9 Months Ex: 30-45 min 3 x week of resistance and plyometrics training with increasing intensity over 9 months Ground rx forces not measured	DXA BMC: TB, LS, FN, TR	NS differences in BMC between groups However, increases in BMC for TB, LS, FN, TR ranged +0.1-2.1% in Ex group	Compliance not reported Potential selection bias

Table 2. Randomized and Non-Randomized Controlled Studies on the Effects of Exercise **o**n Bone Indices in Youth
Ex: exercise group; Con: control group; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: volumetric BMD; BA: bone area; BMAD: bone mineral apparent density (BMD adjusted for BA); TB: total body; LS: lumbar spine; FN: femoral neck; NN: narrow neck; PF: proximal femur;, WT: wards triangle; TR: trochanter; SXA: single energy x-ray absorptiometry; DXA: dual energy x-ray absorptiometry; DPA: dual photon absorptiometry; QCT: qualitative computed tomography, pQCT; peripheral QCT; HSA: hip structural analysis; QUS: quantitative ultrasound; SOS: speed of sound; SM: section modulus; CSMI: cross-sectional moment of inertia; IBS/BSI: index of bone structural strength; SSI: strength strain index; BUA: broadband ultrasound attenuations; OC: osteocalcin; BSAP: bone-specific alkaline phosphatise; CICP: c-terminal procollagen peptide; PYD: deoxypyridinoline; Ca: calcium; Rx: reaction; BW: body weight; PE: physical education; TS: Tanner stage; Pl: Placebo; Grps: Groups; NS: no significant.

3.2 Early pubertal interventions

Eighty-three percent of the PA interventions were capable of creating a positive effect on bone strength parameters in pubertal boys and girls. Study durations ranged from 3 months to 4 years, with both the average and median duration being 12 months. The percent gains in bone ranged anywhere between 1.3-15%; again depending on the measurement location and the technique employed. Of the 16 studies conducted in this group 12 utilized DXA, 3 pQCT, 2 QUS, and 1 SXA. Three of the DXA studies also conducted HSA with 2 of the overall studies employing more than one technique to assess experimental effects on bone. The largest improvements in bone for girls was a 10.3% change in aBMD at the FN following 10 months of a mixed program using jumping, weight bearing exercise and weight training (Morris et al, 1997). This large improvement, however, could be the result of a potential selection bias. In boys, the greatest improvements were in the double digits at 10%, 11%, 14% and 15% for LS aBMD, calcaneal SOS, FN aBMD and vBMD, respectively (Sundberg et al., 2001). These finding in boys were demonstrated after 4 years of increased physical education classes that involved a mixed program of weight bearing and jumping activities. In addition to a PA intervention, 2 of the studies also employed a calcium intervention (Courteix et al., 2005; Iuliano-Burns et al., 2003). These studies (Courteix et al., 2005; Iuliano-Burns et al., 2003) demonstrated calcium supplementation in addition to PA can elicit greater responses in bone than with exercise alone, highlighting the importance of monitoring calcium intake during intervention studies particularly during puberty.

The number of interventions conducted in boys and girls was not equal as it was in the prepubertal group making the discussion on gender differences and effects of PA on bone in this group problematic. Three studies in early pubertal children by the same author (Macdonald et al., 2007, 2008, 2009) incorporated 16 months of 60 minute weekly classroom PA including a bone building program of 5-36 jumps per day 4 times a week. Using pQCT, DXA and HSA these studies demonstrated no significant changes in bone strength in the tibia, but improvements in tibial geometry and bending resistance in boys (Macdonald et al., 2007, 2009). Boys also experienced improvements in lumbar spine BMC and whole body BMC, with girls seeing increases in section modulus (a measure of bending resistance) of the femoral neck (Macdonald et al., 2008). These results imply there may be gender differences in the properties of bone that improve following an exercise intervention. There are 3 reasons why the trends shown by Macdonald et al. (2007, 2009) failed to reach significance. Firstly, there was an uneven distribution of sample size, maturity status and gender between groups making some of the groups underpowered. Secondly, as ground reaction forces were not reported it is possible that external loads applied during the intervention was not high enough to instigate a loading response in bone. Third and most likely, the benefits of the jumping intervention could have been attenuated due to the low compliance to the program. In fact, Macdonald et al. (2008) reported significant findings for individuals with 80% compliance. This notion is supported by 3 studies that (MacKelvie et al., 2001, 2003; Petit et al., 2002) demonstrated improvements in BMC, aBMD and vBMD in girls following a shorter jumping program (7 months) eliciting larger ground reaction forces (3.5-5 x body weight) and for whom study compliance was 80% (MacKelvie et al., 2001).

Not only does it appear that larger loading responses are needed to elicit positive changes in bone, but also the way in which that load is applied to bone matters. A large number of studies (69%) employed specific jumping exercises as part of their intervention demonstrating that short, irregular, diverse large loads at varying times of the day are

sufficient to instigate bone responses (Heinonen et al., 2000; MacKelvie et al., 2001; McKay et al. 2005, Meyer et al., 2011; Petit et al., 2002). Unlike the studies conducted in prepubertal youth, interventions prescribing weight bearing activities do not need to be conducted over long periods of time to see similar responses in bone. Barbeau et al. (2007), Courteix et al. (2005), and Morris et al. (1997) demonstrated such improvements in 7-12 months time. Interventions in which there were no improvements in bone parameters attributed this to higher levels of leisure PA in the non-experimental groups, increased bone mass at baseline, and earlier menarcheal status (Petit et al., 2002; Sundberg et al., 2001). All of these factors would contribute to bone indices being elevated prior to the intervention allowing for only small changes to occur and in turn masking any effects of the intervention program.

3.3 Pubertal interventions

The fewest PA interventions were conducted in pubertal youth, with all 7 involving girls and 1 including boys. The types of interventions included resistance training (Blimkie et al., 1996; Nichols et al., 2001; Witzke & Snow, 2002), jumping trials (Weeks et al., 2008), and those with a variety of different weight-bearing activities (Heinonen et al., 2000, Schneider et al., 2007, Stear et al., 2003). DXA was the predominant method used to asses bone in this population, with one study using DPA (Blimkie et al., 1996). Three of the studies that used DXA also used an alternate method such as pQCT (Heinonen et al. 2000), QUS (Weeks et al., 2008) and serum biochemical markers of bone turnover (Schneider et al., 2007). Half of the trials demonstrated significant changes (0.7-4.9%) in bone following their interventions, with 3 of the studies demonstrating non-significant trends (Schneider et al., 2007; Weeks et al., 2008, Witzke & Snow, 2002). Of those studies that reported significant trends, one included both an exercise and calcium intervention and observed bone mineral advantages at the femoral neck, lumbar spine and total body in adolescent girls receiving both interventions (Stear et al., 2003). Albeit the combination of calcium and exercise generated greater improvements, those girls receiving just the exercise also demonstrated significant changes at the hip. Schneider et al. (2007) provided all pubertal girls with 500mg of calcium per day and unlike Stear et al. (2003) only observed significant changes in thoracic BMC despite improved trends in BMD and markers of bone turnover. It is possible that these results failed to reach significance as the intervention by Schneider et al. (2007) was shorter in duration than Stear et al. (2003), 10 vs. 15.5 months respectively. Moreover, as everyone in Schneider et al.'s (2007) study was taking calcium the room for improvements may have been smaller than Stear et al.'s (2003) who observed the greatest differences between exercising calcium takers and non- exercising non-calcium consuming controls. Regardless of these discrepancies, the one thing that is clear from these two studies and those described in the early pubertal section (Courteix et al., 2005; Iuliano-Burns et al., 2003), is that calcium is important to bone health and its use during PA interventions will greatly affect results.

Three investigations of the effects of resistance training on bone mineral accrual in pubertal girls were completed, with only 1 reporting significant changes in bone indices (Nichols et al., 2001). A major difference between the studies that did not find significant changes (Blimkie et al., 1996; Witzke & Snow, 2002) and the one that did (Nichols et al., 2001) was the duration of the intervention trial. It appears that with resistance training a longer trial of approximately 15 months is necessary to demonstrate significant improvements in bone, similarly to the 15.5 months of WBPA in Stear et al. (2003). In addition to resistance training Witzke & Snow (2002) used plyometric training and the utilization of this may have resulted

in the strong non-significant trends, demonstrating that perhaps shorter trials that include ground reaction forces can be efficacious at improving bone. Results from studies examining jumping trials (Heinonen et al., 2000; Weeks et al., 2008) 8-9 months in duration have been ambiguous. Heinonen et al. (2000) failed to measure significant changes in bone; however, Weeks et al. (2008) did observe improved total body BMC in pubertal boys but not girls. Interestingly, Weeks et al. (2008) did measure large percent changes, albeit non-significant trends, in many different parameters of bone strength in both boys and girls. These trends could be the result of the greater ground reaction forces used in this study compared to that of Heinonen et al. (2000) and could possibly have reached significant if the length of the trial were longer. A common theme in all of these studies not having significant findings or 'almost' measuring differences is poor compliance. If it were not for the issues with compliance, there is a large probability these studies would have found significant results.
Another important factor as to why very few studies reported changes in pubertal youth is due to how bone is accrued in this maturity group. According to Bailey et al. (1996, 1999) peak velocity of BMC accrual for the whole body occurs approximately 0.7-1 year after peak linear growth around the time of menarche, which corresponds to approximately 12-13 years of age in girls. The pubertal girls in the 7 studies reviewed were between the ages of 13 and 18, putting them after the point of peak BMC velocity accrual where the velocity at which they are accruing bone is actually decreasing. The schematic representation of PBM and the rate at which bone mass is accrued over time resembles a dose response curve. It would appear that the pubertal girls in these studies are nearing their PBM, putting them near the plateau of the accrual process, and therefore both the rate and amount of BMC that can be accrued during this time is less. As a result, detecting significant changes will be difficult. Just because these percent gains are small and non-significant statistically does not mean that they are not meaningful. Turner and Robling (2003) demonstrated that a 5.4% and 6.9% gain in aBMD and BMC respectively, translated into a 64% and 94% increase in the amount of force and energy a bone could absorb before failure. This suggests that even small changes in bone mass, which are marginally detectable by DXA can significantly improve bone strength. Therefore a little bone goes a long way.

4. Discussion

4.1 The window of opportunity for bone adaptations

The early pubertal period may be the best time to generate skeletal adaptations to PA. Studies conducted in more than one maturity group demonstrated positive bone gains in early pubertal girls with no significant increases in prepubertal (MacKelvie et al., 2001; MacKelvie et al., 2003; Petite et al., 2002) or pubertal (Heinonen et al., 2000) girls. When reviewing all of the intervention studies the greatest gains in bone on average, regardless of sex, skeletal location and type of activity used, was during the early pubertal years. These results are more definitive in girls as a larger proportion of intervention studies have been conducted on females across puberty, with the sample of boys decreasing with maturity. Despite this trend, longer duration intervention studies where boys most likely transitioned from pre- to early puberty also demonstrate larger gains in bone than in just prepubertal boys (MacKelvie et al., 2004). Larger skeletal gains were also observed in interventions trials that supplemented with calcium during early puberty (Courteix et al., 2005; Iuliano-Burns et al., 2003) compared to those supplementing in prepubertal (Bass et al., 2007) and pubertal

(Schneider et al., 2007; Stear et al., 2003) stages. Moreover, the velocity for BMC accrual is highest in early puberty prior to menarche (in girls) (Bailey et al., 1996, 1997; Cadogan et al., 1998, after which accrual rates decrease with age plateauing in late adolescence upon achieving PBM (Davies et al., 2005). Therefore, the 'window of opportunity' to impart the largest influences on bone development may be during early puberty.

4.2 Optimal physical activity interventions for bone adaptations

Based on our systematic review of the literature we can deduce that regular exercise can be an effective way to improve bone density, size, and shape; in turn improving the mechanical strength of bone. With the variability in the types of interventions used and how they were employed there is no clear consensus on exactly how we should prescribe exercise in order to see the greatest returns in terms of bone health. However, in reviewing the literature, regardless of pubertal stage, the duration of the trial and the intensity in which it was employed appeared to matter. If interventions were short in duration (8-10 months) those that utilized jumping activities with high ground reaction forces received the most positive results (Bass et al. 2007; Fuchs et al., 2001; MacKelvie et al., 2001, 2002; McKay et al., 2005; Petit et al., 2002; Weeks et al., 2008). If weight bearing PA or resistance training was utilized the length of the intervention needed to be longer (10-24 months depending on maturity), in order to see significant gains in bone (Alwis et al., 2008a; Courteix et al., 2005; Linden et al., 2006, 2007; Morris et al., 1997; Nichols et al., 2001; Stear et al., 2003; Valdimarsson et al., 2006). In terms of frequency of exercise, Turner & Robling (2003) suggest it is better to shorten each individual exercise session than to reduce the number of sessions, as jump training has been shown to improve BMC when performed at least 3 time per week but not when reduced to 2 time per week, with gains increasing up to 5 days a week with 2 shorter session in one day. This is reflected in the interventions reviewed with significant gains in bone indices being observed in trials occurring 3-5 times per week. The most recent intervention study reviewed (Meyer et al., 2011) is a good example of these last two concepts by demonstrating that a variety of different activities in one intervention at random times of the day can be effective in eliciting bone gains. Therefore, PA is beneficial for bone health and irregular activities utilizing jump and resisting training to weight bearing activities are some of the best ways to elicit an adaptive response in bone. Not only is the variety beneficial for bone but it can also help to alleviate the boredom that accompanies exercise regimens. Remember that in terms of bone change really is good!

4.3 Methodological issues

DXA was the technique most often used in the PA intervention trials reviewed, and was used to measure BMC and BMD in various skeletal regions of the body. However, BMD assessed using DXA is an estimation of 'true' bone density and the areal density that is expressed is affected by bone size making it difficult to interpret, evaluate and compare BMD in the growing years when there are considerable changes to the size and shape of bone in children (Bailey et al., 1996; Fulkerson et al., 2004; Gordon, 2003; Schoenau et al., 2004). Moreover aBMD is a surrogate measure for bone strength and even though BMC and BMD are related to bone strength inferring information regarding strength from studies using these measures can be misleading. This fact is represented in the many studies citing increases in BMD and BMC that were not always significant. It is possible that DXA may not be sensitive enough to detect small changes in bone particularly at a time in development

when small changes are difficult to come by, like later in puberty when the rate of BMC accrual is decreasing. However, even these small detectable changes in bone mass using DXA can signify improvements in bone strength most likely by favourably altering bone geometry (Turner & Robling, 2003). Therefore the best parameter for assessing the effectiveness of PA interventions on bone would be to use a technique that includes measures of bone strength but also bone shape and size.

pQCT is a method that can be used to detect true vBMD, bone strength, shape and size. Unfortunately, only 5 of the studies that we reviewed utilized this method. An advantage of using pQCT to compare bone structural differences is that it has the capability to demonstrate bone strength adaptations in bone size via changes in cortical thickness or area through investigation of periosteal or endocortical expansion (Haapasalo et al., 2000; Kontulainen et al., 2002; Nikander et al., 2009). Moreover, these measurements indirectly provide an idea of the dynamic course of bone and how bone is metabolized to infer strength. However, to date only 1 study has directly measured biochemical markers of bone turnover in response to a PA intervention (Schneider et al., 2007). Measuring bone turnover would allow for detection of potential exercise effects sooner, as gains in bone markers have been demonstrated after 8 weeks of resistance training in women 20 years of age (Lester et al., 2009). Moreover, reference values for many of the markers have been set within the literature allowing for comparison across studies; something that is difficult to do for static measures of bone as the standards and definitions defining low bone mass are available only for postmenopausal women and not youth.

One way of avoiding this issue is to cease relating bone mass and strength to age, and relate it instead to muscle function (Schoenau & Fricke, 2008). This new methodological concept is based on the thought that the critical property of bone is strength rather than weight and that what influences bone strength are the mechanical loads it must endure either through PA or muscle contraction. Regardless of the mode of mechanical load the stability of the bone must be adapted to muscle strength, in a sense creating a functional muscle-bone unit (Schoenau & Fricke, 2008). Such an analysis removes the concept of a 'peak bone mass', which in fact is something we are not capable of measuring for an individual. Instead this approach allows for determination and comparison of bone deficits irrespective of age as bone strength is related to the strength and function of muscle (Schoenau & Fricke, 2008). Moreover, this approach moves away from looking at bone as a separate entity but as functionally linked system.

4.4 Psycho-social factors

It is also important to consider the psycho-social factors that are believed to affect bone health; these include osteoporosis beliefs, knowledge and practises. Women's willingness to adopt healthy behaviors depends on their level of knowledge of osteoporosis (Cook et al., 1991; Jamal et al., 1999). Majority of research examining calcium intake and PA with respect to osteoporosis knowledge and beliefs, and as preventative behaviours have been investigated in post menopausal women (Tudor-Locke & McColl, 2000). A few researchers have examined these criteria in younger women (Kasper et al., 1994, 2001; Wallace, 2002), let alone in adolescents (Anderson et al., 2005; Schrader et al., 2005). A lack of knowledge about osteoporosis risk factors (insufficient calcium intake and daily PA), as well as perceptions of low risk for developing osteoporosis, has been reported among college women (Kasper et al., 2001) and adolescent females (Anderson et al., 2005). Moreover, studies have suggested

exercise self-efficacy and barriers to exercise are the best predictors of weight bearing exercise and dietary intake (Wallace, 2002), with educational interventions targeting youth demonstrating improvements in bone health knowledge, increases in intake of calcium rich foods and calcium self-efficacy (Schrader et al., 2005; Sharma et al., 2010). Therefore, knowing which factors will help children and adolescents adopt healthy 'bone' behaviors is important to making the exercise interventions we reviewed a reality.

Based on our literature review we know that structured and controlled PA interventions are effective in eliciting bone gains in youth. In order for youth to get involved in osteoporosis preventative behaviors such as PA they need to be able intervene in their daily lives on their own. McWannell et al. (2008) conducted a study to determine whether a structured high impact exercise program would be more effective in improving BMC and BMD than a lifestyle intervention program promoting PA in middle school children 10-11 years of age. This study demonstrated that the structured high impact PA program significantly improved total body BMC and BMD compared to controls after 9 weeks, with the lifestyle intervention seeing insignificant trends for bone gains. Moreover, a health plan-based lifestyle intervention designed at improving both diet and PA in adolescent girls outside of school demonstrated significant improvements in BMD and bone metabolism due to greater consumption of calcium and vitamin D (DeBar et al., 2006). However, when a larger focus was placed on PA and the adolescent girls taught how to properly conduct exercises a self-led PA program proved to be just as significant in improving bone strength parameters as a structured teacher-led PA program (Murphy et al., 2006). More importantly, those girls involved in the self-led PA program continued to exercise after the intervention had ceased, whereas the teach-led group did not. Therefore, it is not only important to get youth physically active in order to improve bone health, it is just as important to develop the personal skills necessary to direct their own activity.

5. Conclusions

With the current growing inactivity and unhealthy dietary habits, the body composition of youth is changing making this systemic review regarding the different types of exercise interventions, those utilizing resistance training vs. ground reaction forces, relevant. For long-term gains, it appears that short-term high-impact exercises undertaken early in childhood (pre and early puberty) if sustained into adulthood has a persistent effect over and beyond that of normal growth and development. Benefits in total body, lumbar spine, thoracic and femoral neck BMC (2.3-4.4%) as well as BMC at the hip (1.4%) have respectively been observed 3 (Gunter et al., 2008b) and 5 years (Gunter et al., 2008) following the jumping intervention by Fuchs et al. (2001). It is therefore redundant in some respect to conduct more PA interventions, unless more advanced techniques of measuring bone are used, as it is apparent from this review that PA in a structured controlled environment is effective in creating positive gains in bone. The next step is to influence change by schools either adopting these activities into their physical education curriculums or providing youth with the tools to administer this change on their own. Therefore, the examination of behavioral, social-psychological variables in addition to physical determinants of skeletal development provides a holistic multi-faceted conceptual framework of bone health that will provide the tools to better disseminate knowledge on positive bone building activities in hopes of creating life-long PA practices.

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Part 6

Prevention and Management of Osteoporosis

21

Osteoporosis, Nutrition and Adolescence

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1. Introduction

Osteoporosis is a worldwide health problem characterized by low bone mineral density and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures. Skeletal bone is maintained by the continuous process of bone remodeling by osteoclasts, cells that break down bone, and osteoblasts, cells that rebuild bone. An imbalance between bone resorption and accrual derives to overall bone loss, which in time leads to osteoporosis (Schettler & Gustafson, 2004). After the third decade of life, the bone mass naturally declines approximately 1-2% per year in women, and 0.3-1% per year in men, leading to losses of 30-50% of their initial bone mass in women and 20-30% in men during their lifetime (Riggs & Melton, 1986). As a consequence, both men and women may develop osteoporosis, maybe the most common chronic disability of postmenopausal women. However, osteoporosis should not be considered solely as adult disease, since bone health must be a lifelong concern, with special focus on the adolescent years (Schettler & Gustafson, 2004). In this sense, Kreipe (1992) suggested that "senile osteoporosis is a pediatric disease".

Osteoporosis in adolescence may be a primary or secondary consequence of diseases or disorders genetic but, in addition, it may be induced by erroneous lifestyle habits, including poor dietary habits, insufficient exposure to sunlight and low physical activities (Campos et al., 2003). The primary forms of osteoporosis in adolescents are relatively rare, and some of them are familiar or genetically determined. In this group may be included the osteogenesis imperfecta, a form of osteoporosis because of bone fragility which is characterized by weak bones that fracture easily, and the idiopathic juvenile osteoporosis, a rare disease associated with a negative calcium balance and characterized by repeated fractures (Bianchi, 2007). The secondary forms of osteoporosis result as a consequence of diseases associated with low bone mass and increased risk of fractures, such as neuromuscular disorders, chronic or endocrine diseases, and inborn errors of metabolism. Moreover, the treatment of some of these diseases may be associated to osteoporosis since several medications such as glucocorticoids, anticoagulants or anticonvulsant drugs can be negatively related to bone metabolism (Bianchi, 2007; Campos et al., 2003). On the other hand, conditions that result in pubertal retardation in adolescents such as anorexia nervosa or amenorrhea induced by exercise, can also be highlighted as causes of osteoporosis in this stage of life.

Although osteoporosis is not common among adolescents, adolescence is a key factor on the development of this disease in the adult age. It has been reported that, probably, the most important factor in the primary prevention of osteoporosis is the attainment of an optimal

peak bone mass during adolescence (Ott, 1990) and, therefore, any factor adversely impacting on bone acquisition during childhood or adolescence can potentially have long-standing detrimental effects on bone health predisposing to osteoporosis (Saggese et al., 2001). Several interconnected factors influence bone mass accumulation during growth, including genetic, hormonal, nutritional and lifestyle factors. Hereditary factors are responsible for around 80% of final peak bone mass, although there are clear suggestions that exogenous factors influence the acquisition of up to 20-25% of bone mass, so that the attainment of 100% of peak bone mass potential may be achieved only by their modulation. According to Ferrari et al. (2000), nutritional and genetic factors may interact to influence bone modeling, affecting bone mineral density (BMD), bone size and architecture, and mineral homeostasis during the years of peak bone mass acquisition. Therefore, together with another lifestyle factors, nutrition during adolescence has an important role in prevention of osteoporosis, and diets consumed during this stage of life should be balanced and equilibrated in order to meet the adolescent's requirements, especially those related to bone health.

2. Bone physiology during adolescence

Adolescence is characterized by an accelerated growth rate associated with rapid muscular, skeletal, and sexual development. During this period 15-25% of the adult size is acquired, approximately 45-50% of total adult skeletal mass is completed and up to 95% of total bone development is completed prior to the age of 18 (Bailey et al., 2000; Henry et al., 2004). Bones are growing in length and width, cortical thickness is increasing, and there is a dramatic increase in bone mass as well as a significant increase in bone density. Bone mineral content during adolescence is more a function of pubertal stage than a function of chronological age (Rico et al., 1993). Before puberty, no substantial gender difference has been reported in bone mass when adjusted for age, nutrition and physical activity. This absence of sex differences in bone mass is maintained until the onset of pubertal maturation, since the gender difference in bone mass is expressed during this period. Bone mineral content accretion accelerates in girls, reflecting the earlier onset of puberty in them, whereas boys have a greater increase in bone mineral content during puberty, resulting in greater values of skeletal maturity (Faulkner et al., 1996). Then, by the age of 10 the mean height-gain velocity is 6 cm/year in girls and increases to an average peak of 9 cm/year by the age of 12. Peak height-gain velocity for boys starts at the age of 12 years (5 cm/year) and reaches a maximum by the age of 14 years (10 cm/year). Mean height gain velocity is close to zero at age 15 in girls, and at age 17 in boys (Matkovic et al., 2004).

Bone is composed by cells (osteoblasts and osteoclasts), minerals (mainly calcium and phosphorus) and organic matrix (collagen and other proteins). Osteoblasts synthesize and mineralize the matrix proteins with hydroxyapatite crystals, whereas osteoclasts promote bone resorption, thus maintaining constant tissue remodeling (Van der sluis & Muinck Keizer-Schrama, 2001). During adolescence two phenomena are produced simultaneously, the synthesis of new bone from growth cartilage due to the process of endochondral ossification, and the modelling-remodelling of previously synthesized bone. The process of bone formation and resorption in the body is continuous, but in adolescents the rate of formation predominates over that of resorption. During puberty, several physiological and endocrine factors have a main role in the accumulation of bone mass. Some of these factors have an important influence on calcium absorption and retention; calcium is the most

important constituent of bone and, therefore, promoting calcium metabolism is a positive factor to enhance bone mineralization. The hormonal changes associated with puberty begin 2–3 years before this period, when an acceleration of growth is observed. The maturation of the hypothalamo-pituitary gonadal axis includes the gonadotropin-releasing hormone (GnRH). At a preprogrammed time in a child's life, there is an increase in the amplitude of GnRH pulses which triggers a cascade of events including increases in the amplitude of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) pulses, followed by a marked elevation in gonadal sex steroidal output, which in turn increases the production of growth hormone (GH) in the hypophysis and of insulin-like growth factor-1 (IGF-1) in different tissues (Mauras et al., 1996). Moreover, sex steroids can act centrally by regulating GH secretion and peripherally modulating GH responsiveness (Figure 1). Higher levels of GH and of sexual steroids during the prepubertal period have a positive influence on bone mineral density and the accumulation of calcium in the skeleton (Krabbe et al., 1979). The effects of GH on bone turnover may be partly mediated by locally produced IGF-1, which is a beneficial factor on skeletal development and bone formation. IGF-1 serum levels increase and reach a maximum during puberty, at 14.5 years in girls and 15.5 years in boys (Juul et al., 1994). This factor seems to be a major regulator of bone growth during childhood and adolescence.

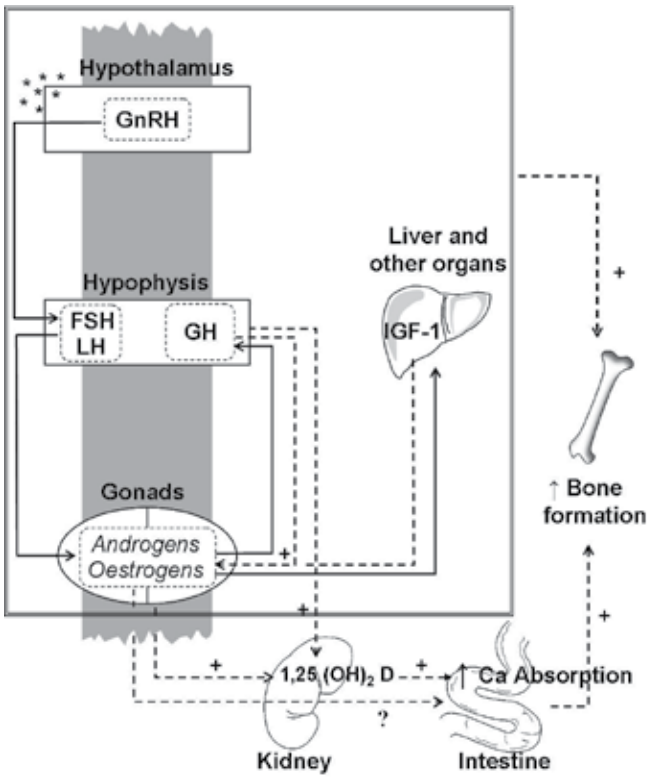

Fig. 1. Hormonal changes during adolescence which are determinants of calcium absorption and bone formation. GnRH: Gonadotropin-relasing hormone. FSH: Folliclestimulating hormone. LH: Luteinizing hormone. GH: Growth hormone. IGF-1: insulin-like growth factor-1. *** : Pulses. —: Hormonal changes. - - - : Effects on calcium absorption and bone formation. Modified from Mesias et al. (2011).

Many of these hormones have an influence on calcium absorption: GH enhances intestinal calcium absorption, increasing 1,25-dihydroxyvitamin D production by stimulating renal 1-*a*-hydroxylase and supports phosphate retention by increasing the renal threshold for phosphate excretion (Bouillon, 1991). The final effect of these actions is the increase of the calcium-phosphate product in the extracellular fluids, which represents a main mechanism for bone matrix mineralization. It is known that GH deficiency may decrease bone turnover, and the balance between bone formation and bone resorption might be uncontrolled. In this sense, it has been demonstrated that children with GH deficiency have reduced bone turnover and bone mass accumulation (Saggese et al., 1995). Several studies have showed that adequate doses and duration of GH replacement therapy are able to increase bone turnover and to achieve bone mineral density values within the normal range (Saggese et al., 1996), suggesting that GH has a fundamental role in the acquisition of peak of bone mass. Moreover, GH, together with IGF-1 stimulates sex steroids secretion (Bouillon, 1991). Both estrogens and androgens influence phospho-calcium metabolism regulating calcium fluxes and bone calcium deposition, increasing calcium absorption and retention (Mauras, 1999). The route by which many of these hormones augment during puberty favoring calcium absorption and bone mass accumulation is across 1,25-dihydroxyvitamin D, the principal enhancer of this mineral absorption at any stage of life but especially in the pediatric period. Nevertheless, although vitamin D is necessary for calcium absorption, on the contrary to the situation found with adults, no relation seems to exist between serum levels of 25-hydroxyvitamin D and calcium absorption in adolescents who are not deficient in this vitamin. This may be because they can adapt to low levels of this vitamin, by increasing calcium absorption independently of the vitamin or, as diverse authors indicate, because during puberty the efficacy of conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D increases to meet the needs for skeletal growth (Abrams et al., 2005).

With the secretion of sex hormones during puberty, bone growth accelerates and bone mass accumulation increases. In females, the accretion rate increases about 4-fold before menarche, although bone mass changes little or even decreases thereafter. In males, bone mass accretion increases approximately 6-fold during puberty with a slower but still marked accretion at many skeletal sites thereafter. In addition, there are gender differences in the porosity of bone between adolescent boys and girls that may reflect greater bone remodelling in boys at this time. As a result of these differences, males have a larger bone size and greater thickness after puberty than females, but there is little difference in volumetric density (Prentice et al., 2006). Estrogens are an important determinant of bone mineral density in girls during puberty; this is confirmed by diverse studies in which girls with an early or regular menarche had higher bone mineral density, while a late menarche or amenorrhea in ballet ballerinas or in patients with anorexia nervosa were related to a limited density and even to fractures (Bachrach et al., 1991; Young et al., 1994). Estrogens can decrease the rate of bone turnover, and inhibit the osteoclastic resorption of bone by affecting bone cell differentiation and function. They also affect the parathyroid hormone (PTH) and, as mentioned previously, vitamin D metabolism. Androgens may also be important determinants of bone density, although studies carried out with animals have shown that estrogens play a more important role than androgens in skeletal mineralization (Frank, 1995). Another factor related to mineral density is serum calcitriol (1,25-dihydroxyvitaminD). It is demonstrated (Ilich et al., 1997) that calcitriol levels can be positively associated with changes in total bone mineral density during pubertal growth,

presumably in response to the high requirements for calcium during this critical phase of skeletal development.

3. Nutritional factors affecting bone development

During adolescence several nutritional factors play a major role in the bone mass gain process and, therefore, some of the nutrients and food components consumed as part of the diet can potentially impact bone accrual during this stage of life. In addition, several nutritional disorders may be associated with osteoporosis.

Dietary factors that may affect bone metabolism include minerals such as calcium, phosphorus and magnesium and a variety of nutrients cofactors such as vitamins D, C and K, and other minerals such as copper, zinc and manganese. In addition, a positive energy balance from macronutrients is important during growth for synthesis of bone. On the other hand, protein comprises most of the nonmineral composition of bone, and an adequate protein intake is essential for bone matrix synthesis (Saggese et al., 2001). Therefore, diet must contribute sufficient and appropriate nutrients to allow, together with healthy lifestyle habits, achieve the maximum genetic potential for bone mass development.

3.1 Calcium

Calcium is the most abundant mineral in the organism and contributes approximately 1-2% to the adult human body weight. About 99% of body calcium is deposited in bone and teeth and, hence, its main function is structural, being essential for optimal growth and development. A dynamic balance exists between calcium in the extracellular medium and that found in bone, and about 500 mg of this mineral enter and depart daily from the bones (Pérez Llamas et al., 2010). The bone acts as a reservoir of calcium to maintain extracellular homeostasis and transfers the mineral if its concentration in blood falls below normal values (9.0-10.2 mg/dl), especially in situations of chronic calcium deficiency resulting from continual inadequate intake or poor intestinal absorption. Therefore, mineral deficiency leads to inadequate mineralization of bone matrix, resulting in rickets in children and adolescents and, along with other risk factors, contributing to possible osteoporosis in adulthood (Mesías et al., 2011).

Calcium requirements vary throughout life; greater needs are shown during periods of intense growth such as childhood and adolescence, during pregnancy and lactation, and also later in life. Among adolescents, a calcium increase is needed as a result of the intensive bone and muscular development. Therefore, adequate calcium intake during growing is essential to reach the optimum peak bone mass, which, as it has been above mentioned, protects against osteoporosis in the adult age (Story & Stang, 2005). Although peak bone mass has a large genetic component, there is evidence that it can be enhanced by increasing calcium consumption. Several studies in children and adolescents have shown that bone mass and bone density increase with calcium dietary supplements, and therefore, providing adequate calcium intake during the formative years is one approach to optimizing peak bone mass (Cromer & Harel, 2000).

Given the high proportion of body calcium present in bone and the importance of this as the major calcium reservoir, the development and maintenance of bone are the major determinants of calcium needs. Therefore, adolescents must consume diets that are balanced and adjusted to their requirements in order to meet calcium recommendations and to obtain the energy and nutrients that promote mineral utilization. Several studies have

demonstrated the importance of considering both food groups and the overall diet in assessing their impact on bone health, which may partially explain the relationship between nutrient intake and bone mineral acquisition in children and adolescents (Heaney, 2004; Seiquer et al., 2008). Milk and dairy products contribute around 70% of total dietary calcium and thus, they are by far the main source of calcium in Western diets (Guéguen & Pointillart, 2000). Addition of these products to the adolescent's diet is the best strategy to meet calcium recommendations and to achieve optimal bone mineralization (Cadogan et al., 1997). Dairy products contribute from 42% of the total calcium consumed by British adolescents (Moynihan et al., 1996) to 70% by Australian (Department of Community Services, 1989), Spanish (Seiquer et al., 2006), or American adolescents (Fiorito et al., 2006). Dietary calcium supplements improves bone mineral accretion by 1-5% among adolescents consuming less than 1000 mg Ca/day, and by up to 10% when supplemental calcium is provided by dairy products (Kerstetter & Insogna, 1995). In this sense, Lyriris et al. (1997) found a correlation between the consumption of dairy products by young adult humans and bone density. Moreover, low milk intake during childhood and adolescence is associated with a greater incidence of fracture among older women (Kalkwarf et al., 2003). Cereals may also constitute an important calcium source, whereas meat, eggs, fish and legumes are minority calcium sources in the diet of adolescents (Seiquer et al., 2008). In addition, drinking water, including mineral water, may provide 6–7% of daily calcium intake (Guéguen & Pointillart, 2000). On the other hand, nutrients found in abundance in fruit and vegetables may be protective for bone health (Jones et al., 2001), as discussed below.

To achieve the maximum peak bone mass during adolescence, it is mandatory a positive calcium balance, i.e., the calcium body retention calculated as intake minus losses (Anderson & Garner, 1996). Usually the calcium balance increase in parallel to the intake, which suggests that the intake of this mineral may limit growth. Dietary calcium during this stage has a direct relationship with bone mineralization and low intake during puberty may limit it (Cadogan et al., 1997; Matkovic et al., 2004). Thus, if calcium intake is below 500 mg/day in childhood, more than 50% of ingested calcium must be retained in order to obtain adequate mineral accretion (Mølgaard et al., 1999). Balance studies carried out in adolescents support that calcium retention is associated with calcium intake, but at intakes up to 1300 mg/day a plateau is reached (Jackman et al., 1997). Therefore, calcium is a threshold nutrient, i.e., at suboptimal intake the body's ability to store calcium as bone tissue is limited by the intake of the mineral, but increasing calcium intake above the body's requirements does not result in further increases of stores (Mesías et al. 2011). At calcium intakes producing optimal bone mass, increasing calcium intake will not result in more bone (Flynn, 2003).

Intestinal calcium absorption varies with age and adapts to different physiological situations, so that when needs are high, mineral absorption becomes more efficient. Puberty is associated with a high rate of dietary calcium absorption, not only in absolute values but in fractional absorption rates or digestibility, in order to satisfy the increased calcium requirements for the intensive adolescent growth (Abrams & Stuff, 1994). To enable an increase in mineral absorption, adolescents have a low rate of calcium fecal excretion. Besides the amount of calcium in the diet, food ingredients are also a critical factor in determining the available calcium for bone development and maintenance. There is therefore a need to identify food components and/or functional food ingredients that optimize calcium absorption and bioavailability. Some components of the diet have been suggested as enhancers or inhibitors of calcium absorption. Thus, phytates found in bran

and most cereals and seeds, oxalates in spinach or walnuts, and tannins in tea, can form insoluble complexes with calcium, thereby reducing its absorption. However, Guéguen and Pointillart (2000) show that these factors only seem to significantly affect calcium balance if the overall diet is unbalanced. Milk and dairy products are considered good sources of this mineral due to their high calcium content and bioavailability (proportion of calcium retained from intake). Around 40% of the calcium from these products may be absorbed due to the particular physico-chemical form of the element and the presence of absorption promoters such as lactose and caseinphosphopeptides. The latter, moreover, can limit the inhibitory effect of other compounds (Guéguen and Pointillart, 2000). Milk nutrients may promote bone mineralization because, in addition of being a major source of calcium, milk provides phosphates, magnesium, proteins, and as yet unidentified nutrients likely to favor bone health (Esterle et al., 2009). Vitamin D is also an essential factor for intestinal calcium absorption and plays a central role in maintaining calcium homeostasis and skeletal integrity. Adequate levels of this vitamin are obtained by suitable intake and sufficient exposure to sunlight, which is the major source of vitamin D in the organism (Bener et al., 2008). On the other hand, certain lipids may favor calcium bioavailability; it has been shown that a high ratio of unsaturated to saturated fatty acids has beneficial effects on calcium absorption (Haag et al., 2003). Fish may be a good source of calcium because on the one hand its protein is as positive as casein in promoting calcium absorption (García-Arias et al., 1994), and on the other hand because omega-3 fatty acid might promote calcium transport (Haag et al., 2003). It should be noted that the positive effect of fish fat in calcium utilization is promoted when it is consumed together with olive oil (Pérez-Granados et al., 2000), as it occurs when fried fish are consumed. Olive oil may be another contributor to enhanced calcium utilization, assays have shown that oleuropein, an olive oil phenolic compound, reduces bone loss (Puel et al., 2004). Studies in humans (Van den Heuvel et al., 1999) and experiments in rats (López et al., 2000) have revealed a positive effect on apparent calcium absorption after consumption of oligofructose. This compound may also diminish the negative effects of phytic acids. A diet rich in cereals, fruits, and vegetables can increase the presence of these prebiotic products in the digestive system, helping to improve calcium absorption in this physiological stage when demands for the mineral are high.

Dietary calcium intake and urinary excretion of calcium seem to be also important determinants of mineral retention in the body. Although in adults urinary calcium may reflect the intake, in adolescents levels of calcium in urine represent obligatory renal losses that are independent of the consumption. Therefore, it can be supposed that only an unavoidable amount of calcium is lost because its use at this stage of life is a matter of priority for the organism. During childhood, urinary calcium excretion doubles, from ~40 mg/day in young children to ~80 mg/day just before puberty. However, during the peak of maximum growth, this value decreases, especially among males. Calcium excretion rises to reach the values of adulthood (100-250 mg/day) by the end of adolescence (Manz et al., 1999; Peacock, 1991). This increase in urinary calcium excretion in late adolescence probably reflects the decreasing needs of the skeleton for calcium (Peacock, 1991). In addition to the physiological status, certain dietary components may affect urinary losses of calcium. Some dietary factors affecting urine losses have a major influence on calcium balance and may even become more important than those influencing the intestinal availability of calcium. Thus, excessive protein intake, particularly animal protein, generally leads to an increase of the calcium lost in urine (Ginty, 2003), although it seems unclear whether protein intake has a negative effect on calcium balance and bone mineralization in children and adolescents.

Independent factors can be related to urinary mineral excretion, but total urinary excretion is determined by the metabolic effect of the overall diet. Nutritional intervention studies have shown that a high intake of fruits and vegetables decreases urinary calcium in adults and adolescents (Tylavsky et al., 2004). In turn, fruits and vegetables provide organic salts of potassium and magnesium that have a buffering effect and consequently decrease urinary calcium. This effect has been demonstrated in adults (Whiting et al., 1997) and in adolescents (Jones et al., 2001). On the contrary, low phosphorus and high sodium and caffeine intake are associated with increased urinary calcium (Kiel et al., 1990; Brunette et al., 1992; Weisinger & Bellorin-Font, 1998). With an adequate diet, calcium bioavailability is favored, reaching values around 36.5% for boys and 29.6% for girls, or even higher when diets provide suitable amounts of the mineral (Bailey et al., 2000; Seiquer et al., 2008). Thus, as mentioned above, the dietary habits of adolescents are an important factor to meet calcium requirements and, consequently, needs for pubertal growth.

3.2 Phosphorus

Together with calcium, phosphorus is essential during adolescence to support the rapid rate of bone accretion that takes place in the adolescent growth spurt. Almost 85% of the body's phosphorus is located in bone as calcium phosphate salt in the form of hydroxyapatite, with a Ca:P molar ratio approximating 1.7:1 and, therefore, this mineral must be present in adequate amounts in the diet to mineralize and maintain the skeleton. Adequate supplies of both minerals are crucial to maximize bone mineral accrual during growth, considered the best strategy to prevent age-related osteoporotic fractures later in life (Weaver, 2000). However, in spite of dietary phosphorus has an important and positive role to play in the development of peak bone mass, it has been suggested that both high and low phosphorus intakes may seriously alter calcium metabolism. On the one hand, excessive amounts of this element may be harmful to bone health and it should be taken into account the low calcium to phosphorus ratio. There is some evidence that increased phosphorus intake depresses ionised calcium leading to an increase in PHT and, hence, a rise in the rate of bone resorption (Calvo et al., 1988). On the other hand, it has been reported that low phosphorus intake is associated with increased urinary calcium (Weisinger & Bellorin-Font, 1998). Other studies have failed to show a deleterious long-term effect of different phosphorus intakes on calcium balance (Heaney & Recker, 1982).

3.3 Magnesium

Total body magnesium content is approximately 25 g, 60-65% of which is found in bone. Part of this magnesium is in equilibrium in an exchangeable way with the extracellular magnesium, and may serve as a reservoir for maintaining a normal extracellular magnesium concentration; so that at reduced plasma concentration, magnesium can be rapidly released from the bone surface and at increased plasma concentrations magnesium remains bound to the surface of bone (Elin, 1994). However, experimental evidences that dietary magnesium influences the development of peak bone mass are scarce.

Magnesium plays a major role in bone and mineral homeostasis and can also directly affect bone cell function as well as influence hydroxyapatite crystal formation and growth. This element is required for matrix and mineral metabolism in the bone through its indispensable role in metabolism of ATP and as a cofactor for over 300 enzymes (Sojka, 1995). Although the requirement for magnesium retention during childhood and adolescence is uncertain, it

is likely to be 5-10 mg/day (Andon et al., 1996) and may increase during the pubertal growth spurt in order to support the more rapid rate of bone formation during this period (Abrams et al., 1997).
The bioavailability of magnesium may be affected by several dietary factors such as phosphorus, calcium, sodium or protein. It is known that high phosphate diets can decrease intestinal magnesium absorption due to the ability of phosphate to bind magnesium (Reinhold et al., 1991), whereas high sodium and calcium intake may result in increased renal magnesium excretion (Kesteloot & Joossens, 1990). In addition, dietary protein may also influence magnesium utilization; magnesium balance is negative when protein intake is less than 30 g/day, due to a high mineral excretion in urine and feces (Hunt & Schofield, 1969) whereas higher protein intakes, around 94 g/day, also may increase renal magnesium excretion (Mahalko et al., 1983), since the acid load increases urinary magnesium excretion (Wong et al., 1986).

3.4 Protein

In addition to calcium, dietary protein represents a nutrient essential for the synthesis of bone matrix. Protein is a major constituent of bone, so adequate protein intake is critical to maintaining bone health. Several studies have demonstrate that a selective deficiency in dietary proteins, without any associated insufficiency in other macronutrients such as total energy, calcium and vitamin D, causes a rapid and marked alteration in bone mass, microarchitecture and strength (Bonjour, 2005). It is known that proteins can stimulate intestinal calcium absorption and enhance IGF-I. Preclinical studies in adult animals have documented that an isocaloric low protein diet reduces IGF-1, induces negative bone balance with both decreased formation and increased resorption, thereby leading to a decline in bone strength (Ammann et al., 2000; Bourrin et al., 2000).
However, the effects of dietary protein intake on bone health are controversial, since it also has been documented that higher protein diets increase urinary calcium, being therefore a risk factor for osteoporosis. Protein intake increases acid production and renal acid excretion due to the releasing of protons during the oxidation of sulfur-containing amino acids such as methionine, cysteine, and cystine. This metabolic acid load might cause the dissolution of bone mineral, which would originate an increased calciuria, resulting in an accelerated loss of bone mineral mass and, thereby, increasing the risk of osteoporotic fracture at long term. The higher content of sulfur-containing amino acids in animal proteins compared with vegetable proteins would lead to increased urinary excretion of calcium and, therefore, to exacerbation of age-related bone loss. Therefore, vegetal proteins might be bone protective whereas animal proteins would be harmful for the acquisition and the maintenance of the bone mineral mass (Sellmeyer et al., 2001). However, the harmful effect of excessive animal protein in skeleton formation seems to be only significant when calcium intake is inadequate (Heaney, 1998).

4. Dietary habits of adolescents related to bone health

4.1 Intake of bone-forming nutrients

Although adequate calcium intake during childhood and adolescence is mandatory, a high percentage of American and European adolescents fail to meet the recommendations of this mineral (Table 1).

	Recommendations	Intakes	
	DRIs[1-2]	Europeans[2-3]	Americans[4-5]
Calcium	1300 mg/day	596-1400 mg/day	793-1081 mg/day
Phosphorus	1250 mg/day	949-1848 mg/day	1093-1533 mg/day
Magnesium	240-410 mg/day	185-360 mg/day	216-284 mg/day
Protein	34-52 g/day	53-127 g/day	78 g/day

References: [1]Institute of Medicine (1997); [2]Institute of Medicine (2005); [3]Lambert et al. (2004); [4]Elmadfa et al. (2005); [5]Rockett et al. (2001); [6]Ervin et al. (2004).

Table 1. Recommendations and intakes of calcium, phosphorus, magnesium and protein among adolescents.

According to Grunbaum et al. (2004), only 11% of female and 23% of male American adolescents drink three or more glasses of milk daily, and only 19% of girls and 52% of boys meet calcium recommendations (Damore et al., 2007). Among Spanish population, 13–14% of boys and 29–40% of girls have inadequate calcium intakes (Serra Majem et al., 2006). In conclusion, calcium content in the adolescents diet frequently fails to meet the body's needs during the growth spurt (Rocket et al., 2001; Lambert et al., 2004; Elmadfa et al., 2005), which, as it has been mentioned before, might have a deleterious effect on the acquisition of the peak bone mass and, therefore, an important repercussion on osteoporosis in adult age.

In recent years the contribution of milk to total beverage intake has significantly decreased among boys and girls because milk has been replaced by carbonated beverages (Vatanparast et al., 2006). Since 1965, milk consumption has decreased by 74%, and consumption of non-citrus juices and carbonated beverages has increased by 118% (Schettler & Gustafson, 2004). Therefore, soft drinks negatively affect bone mineralization because they are associated with lower milk consumption. But, moreover, a further negative effect concerns their phosphorus and caffeine content; the phosphoric acid content of soft drinks may limit calcium absorption and contribute to bone loss increasing bone resorption and fracture risk (Wyshak & Frisch, 1994; Wyshak, 2000), whereas caffeine has been associated with reduced bone mineral density and increased fracture risk (Kiel et al., 1990). Both effects have been demonstrated in children who frequently consume cola drinks (Heaney et al., 2000). However, several studies have reported that the negative impact of soft drink on bone mass is observed in adolescent girls but not in boys (Whiting et al., 2001; McGartland et al., 2003), which implies that bone accrual mechanisms in adolescent girls have more vulnerable conditions.

As it can be observed in Table 1, a certain proportion of American and European adolescents also fail to meet phosphorus recommendations. However, the overall phosphorus intake has increased during the last years as a consequence of the changing dietary habits of adolescents, on the one hand, the greater carbonated beverages consumption and, on the other hand, the increased intake of processed and fast foods, very frequently consumed among this age group. Manufactured and fast foods have a high content of phosphorus-containing additives, used to preserve moisture or color, to emulsify ingredients, enhance flavor, and to stabilize foods. According to Coates et al. (2005), these additives are the most rapidly growing source of dietary phosphorus over the last two decades and may contribute to one-third of overall phosphorus intake in the general population. The high phosphorus

intake, therefore, should be taken into account due to its negative association with calcium metabolism and, in addition, with intestinal magnesium absorption.
Regarding magnesium intake, many adolescents usually do not reach mineral recommendations (Table 1), but, moreover, dietary factors can affect metabolism and excretion of this mineral, as mentioned. Abrams et al. (1997) reported that among boys and girls aged 9-14 years consuming RDA magnesium intakes, a significant number of them were in negative magnesium balance. This negative balance appeared to be related primarily to urinary excretion of magnesium, probably affected by dietary factors, which might affect to mineral homeostasis and bone formation.
On the other hand, as it is usual among population of Western countries, the protein intake among adolescents is above the recommendations, with special contribution of animal proteins (García-Closas et al., 2006). High protein intake may be related to increased urinary calcium and magnesium excretion but, moreover, the low consumption of fruits and vegetables among adolescents decreases the buffering effect above-mentioned, increasing the negative effects of protein on mineral utilization and, therefore, on bone health. Several authors have shown the link between fruits and vegetables and peak bone mass acquisition in boys and girls (Tylavsky et al., 2004). Whiting et al. (2004) confirm that girls consuming adequate amounts of this food group show a greater bone mineral trajectory than those consuming fewer than 5 servings/day. In the same way, subjects with an intake of 10 servings per day of fruits and vegetables presented a higher total body bone mineral content than did those consuming 1 serving/day (Vatanparast et al., 2005). Moreover, fruit and vegetables provide vitamin K, which is an essential cofactor for osteoblastic activity (Feskanich et al., 1999) and natural antioxidants like phytestrogens, which seem to play a role in bone metabolism. Phytestrogens, like estrogens, stimulate human osteoblasts and modulate osteoclast activity, thus preventing bone resorption (Chiechi & Micheli, 2005). Therefore, a diminution of fruits and vegetable consumption, frequently observed in adolescents, avoid the protector effect of these types of foods on bone.
It is well known that the dietary habits of adolescents have changed in recent decades and that there is a tendency to a higher consumption of soft drinks, snacks, bakery products, and fast foods, which, particularly, has increased from 2% of total energy in the late 1970s to 10% of total energy in the mid-1990s (Guthrie et al., 2002; Libuda et al., 2008). As it has been mentioned, the consumption of these kinds of foods may be associated to lower intake of fruits and dairy products and greater intake of phosphorus due to high phosphorus-containing additives, which implies negative effects on calcium utilization. Together with phosphate salts, processed foods are also rich in sodium salts-containing additives (He et al., 2008) which, certainly, increases sodium intake. It is known that average sodium intakes in children and adolescents well exceed nutritional needs, overcoming even 3.5 g/day (Falkner & Michel, 1997). Since the elevated consumption of sodium also damages bone by increasing the urinary calcium excretion and decreasing calcium absorption (Brunette et al., 1992), reducing sodium intake should be seriously considered among adolescents.

4.2 Intake of Maillard reaction products

The Maillard reaction, also termed nonenzymatic browning reaction, is usually developed in processed and fast foods, since it commonly occurs during the thermal processing of foods rich in proteins and sugars or fats, producing colored compounds that contribute to the aroma, color, and flavor of cooked foods. Controlled browning is therefore pursued through

many food technologic and domestic processes such as roasting, baking, frying and even re-heating, aimed at promoting consumer acceptance (Ames, 1998). Thus, the Maillard reaction products (MRP) are widely consumed as a part of the human diet, especially among adolescents, according to their dietary habits and the high content of snacks and fast foods in their diets (Delgado-Andrade et al., 2007). In addition to their sensory properties, MRP is associated with certain positive biological effects, such as antioxidant activity (Seiquer et al., 2008), but at the same time with negative actions, including degradation of nutritional protein quality (Alkanhal et al., 2001) and modifications in vitamins (O'Brien and Morrissey, 1989) or mineral availability (Navarro, 2003).

Our research group has realized several studies with the objective of comparing the effects of diets with different MRP contents on the utilization of dietary protein and on mineral availability in adolescents. In a 2-period crossover trial, a group of healthy male adolescents aged 11-14 years consumed two types of diets, both balanced and varied and with the same nutrient composition, but with different content in MRP. The first one was a MRP-poor diet, free, as far as possible, of foods in which the Maillard reaction develops during cooking practices or foods that naturally contain these products whereas the second one was a MRP-rich diet, high, as far as possible, in processed foods with an evident development of browning. The utilization of the different nutrients by the subjects under consumption of the different diets was measured. The effects of the high consumption of MRP on nutrient concerning bone formation are summarized in Table 2.

Factor	Absorption	Retention	Digestibility	Bioavailability	References
Calcium	=	=	=	=	Mesías et al., 2009
Phosphorus	↓↓	↓	↓↓	↓	Delgado-Andrade et al., 2011
Protein	↓↓	↓	↓↓	↓	Seiquer et al., 2006

Table 2. Effects of a MRP-rich diet on protein and mineral availability compared with a MRP-poor diet in adolescents. =: non effect; ↓↓: effect statistically significant; ↓: effect non significant statistically.

Absorption and digestibility of nitrogen were significantly lower when subjects consumed the MRP-rich diet than the MRP-poor diet, whereas negative effects on the protein balance did not reach statistical significance. It was deduced that the consumption of a diet rich in browning products negatively affects protein digestibility (Seiquer et al., 2006). Regarding minerals, it is known that MRP may behave as anionic polymers that chelate metal cations, affecting mineral solubility at intestinal conditions and mineral availability (Navarro, 2003). In our assays we observed that high MRP intake has no apparent effects on dietary calcium bioavailability in adolescent, although possible metabolic changes cannot be discounted, as a lower deoxypyridinoline urinary excretion was observed with MRP-rich diet consumption, which may be related to decreased bone turnover at this age (Mesías et al., 2009). On the contrary, this diet had clear negative effects on dietary phosphorus absorption, tending to decrease the phosphorus balance (Delgado-Andrade et al., 2011). In a similar way, MRP-rich diet did not affect zinc but negatively affected copper bioavailability (unpublished data). It should be borne in mind that the food habits of adolescents are changing toward monotonous and unbalanced diets with a considerable MRP content, and

an increasing proportion of total energy intake is obtained from fast food and snacks, as mentioned. In these conditions, the effects observed in our studies could be aggravated. In view of the current dietary habits of adolescents, and the well-established relationship between protein and mineral deficiency with bone formation during the growth spurt, and, consequently, with osteoporosis in the adult period, it seems of special interest to take into account the possible long-term effects of dietary MRP on bone health.

4.3 The Mediterranean diet

The Mediterranean diet has been proposed as one of the healthiest dietary models and its health benefits have been demonstrated in a large number of studies. Moreover, the Mediterranean diet incorporates practically all the factors that may positively influence bone health. However, current dietary patterns are considerably far from the characteristics of the Mediterranean diet, particularly among adolescents due to the increased habit of eating away from home and the higher consumption of snacks and fast foods.

This diet is characterized by moderate levels of animal protein, abundant fresh fruits and vegetables, cereals and fish, and little saturated fat. Olive oil is used as the main dietary fat.

Our research group has also studied the effects of a varied diet based on Mediterranean patterns on the utilization and availability of nutrients essential for bone formation, such as protein and minerals, among adolescents. A summary of the results obtained in our studies, comparing with those obtained when the subjects are under their own diets, is shown in Table 3.

Factor	Absorption	Retention	Digestibility	Bioavailability	References
Calcium	↑↑	↑↑	↑	↑↑	Seiquer et al., 2008
Phosphorus	↑	↑↑	=	↑↑	unpublished data
Protein	=	↑↑	=	↑↑	unpublished data

Table 3. Effects of a varied diet based on Mediterranean patterns on protein and mineral availability in adolescents. =: non effect; ↑↑: effect statistically significant; ↑: effect non significant statistically.

It has been shown that dietary calcium utilization during adolescence may be greatly improved by a diet based on the Mediterranean patterns (Seiquer et al., 2008). Compared with the consumption of their habitual diets, adolescents significantly increased the absorption and retention of the dietary calcium, and, as a consequence, calcium utilization efficiency was significantly improved when subjects consumed the Mediterranean diet. In a similar way, after this same diet consumption, adolescents significantly increased phosphorus and protein retention and bioavailability (unpublished data). Therefore, a diet with sufficient calcium, phosphorus and protein and based on the Mediterranean diet patterns is advantageous for bone formation during periods of intense growth, such as the adolescence, when factors affecting bone health will be determinants for the development of osteoporosis later in life.

4.4 Lifestyle: Physical activity and others

Together with eating behaviors, weight-bearing physical activity is a modifiable pattern that could be of potential importance in ensuring that the maximum genetic potential for bone

mass is achieved (Matkovic et al., 1990). This kind of activity could be defined as physical activity in which gravity exerts force on bones or any activity done standing up (e.g., walking or jumping), not including activities that involve only resistance or that are done sitting down (e.g., bicycling or swimming). In general, studies support the view that moderate weight-bearing activity has a more positive effect on bone mass than do non-weight-bearing activities, which impose minimal physical strain on bone (Cromer & Harel, 2000). Several studies have shown that both exercise and calcium interventions have an overall beneficial impact on bone acquisition during childhood and adolescence (Stear et al., 2003). According to Anderson (2001), physical activities during the critical growing years make important contributions to the accrual of bone mass, perhaps independently of calcium intake. In this way, Nickols-Richardson et al. (1999) reported that a relatively low calcium intake may be compensated by regular physical activities in the accrual of peak bone mass.

The current lifestyle habits of many adolescents, based mainly in inadequate dietary intake and insufficient physical activity, have resulted in an increased level of overweight and obesity particularly in Western countries. Overweight among children has been related with an increased incidence of fractures (Greer & Krebs, 2006), which is probably explained by the fact that calcium intake is negatively correlated with body fat percentage and body mass index during childhood (Carruth and Skinner, 2001; Skinner et al., 2003). The inverse relationship between calcium intake and fatty tissue gain has been recently confirmed by Lederer et al. (2009) in male adolescents. It has been shown that overweight children have a lower bone mass and bone area relative to their body weight than do children with a healthy body weight, which may predispose them to fractures (Goulding et al., 2000). Trends in diet and exercise coupled with an increasingly aging population indicate that the incidence of osteoporosis will triple by the year of 2040 (Schettler & Gustafson, 2004), with the result that both diet and physical activity are considered to be important and complementary factors to prevent this disease.

Other fact that should be taken into account among adolescents in order to prevent osteoporosis is tobacco and alcohol consumption. It has been reported that the prevalence of concurrent alcohol and tobacco use among European and American adolescents comes to 20-25% (Anthony and Echeagaray-Wagner, 2000; Schmid et al., 2007). Clinical findings indicate that there is an inverse relationship between bone mineral density and smoking, due to its negative effect on calcium absorption and estrogen metabolism (Bailey et al., 2000; Valimaki et al., 1994). Moreover, heavy alcohol consumption hinders calcium absorption and damages bone cells (Bennet, 1995). On the other hand anorexia nervosa, a dangerous disease in adolescent girls at the time of the initial peak bone mass formation, is known to be associated with low bone mass content, even in short duration cases, effects that may persist even after recovery (Misra et al., 2004; Winston et al., 2008). It has been reported that adult women with anorexia nervosa initiated during adolescence have lower bone mass than those with adult onset anorexia nervosa (Biller et al., 1989). This disease includes alterations of the GH-IGF-1 axis and, moreover, both hypogonadism and the cortisol excess associated to it may contribute to the development of osteopenia and osteoporosis (Misra et al., 2005a; 2005b).

5. Conclusion

Prevention of osteoporosis in adulthood begins in childhood. Therefore, in the fight against osteoporosis, modifiable factors such as dietary and lifestyle patterns should be taken into account from the beginning of life. Adolescence is a phase of particular interest for the

prevention of osteoporosis due, on the one hand, to the special dietary habits of adolescents, and on the other hand, to the great development and acquisition of the skeletal mass during this stage of life. Not only meeting adequate intakes of minerals and protein is required during adolescence, but, moreover, the composition of the whole diet will be determinant for consecution of the maximum genetic potential for growth and bone development. Diet, therefore, must contribute sufficient and appropriate nutrients to allow, together with healthy lifestyle habits including physical activity, the maximum bone mass development genetically programmed, which will be the best strategy in the prevention of osteoporosis.
Due to the progressive incidence of osteoporosis in Western countries, intervention policies for this important and vulnerable sector of the population should be aimed to promote the consumption of adjusted and balanced diets among adolescents, since the adequate utilization of nutrients, together with exercise, will benefit their present and future health.

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22

Rehabilitation in Osteoporosis

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1. Introduction

Osteoporosis is a metabolic bone disease usually occurring with increasing age and is defined as a skeletal disorder characterized by reduced bone mineral density and strength. Osteoporosis is characterized as the "silent disease" because is painless until the first occurrence of a fracture and thus remain unnoticed. The first symptom of osteoporosis is the bone fracture with a preference of distal radius or proximal humerus fracture, vertebral collapse and femoral neck fractures beyond the 50th, 60th and 70-75th year respectively (Pfeifer et al., 2005). This phase of the disease is characterized by acute pain. Moreover, vertebral fractures cause acute musculoskeletal pain in the back in the acute phase of the fracture and chronic pain resulting from the associated skeletal deformity, joint incongruity, and tension on muscles and tendons, leading to disability. In generally osteoporosis represents one of the main causes of back pain in postmenopausal women because of common clinical or subclinical vertebral fractures causing back pain. On the other hand, in the same population, no osteoporotic vertebral deformities are seen as often as osteoporotic ones, and back pain was found to be mostly due to degenerative disorders of the spine in women above 60 years (Dionyssiotis, 2010b).

Osteoporosis is a disease that predominantly affects postmenopausal women and older people although in individual cases could concern people in younger age i.e. in the juvenile form, mainly men with idiopathic osteoporosis, pregnancy-associated osteoporosis, the form of secondary osteoporosis in young steroid-treated patients with chronic inflammatory diseases etc. The goals of rehabilitation are changing depending on the stage of disease. In the acute phase of a vertebral body collapse the therapy is to the relief pain by a limited period of bed rest, local and systemic analgesia, bracing, physical therapy, education with proper exercises and instructions according to daily living activities in order to mobilise the patient with safety. Rehabilitation after surgical stabilization of a hip fracture is crucial in order to optimize post-injury mobility and the functional recovery of the patient, restore pre-fracture function and avoid long-term institutionalization. Most evidence-based guidelines suggesting possible treatments and rehabilitation pathways for hip fracture patients, agree that it would be best if they underwent multidisciplinary rehabilitation (Dionyssiotis et al., 2008a).

In prevention and management of osteoporosis modern rehabilitation medicine should not only focus on bone ignoring muscular strength and balance. These elements are directly related to the disease offering protection against predisposing a person to an increased risk of falls and fall-related fracture. An extensive research in the area of pharmacological

treatment is ongoing. Pharmacologic treatment increases bone strength, but has no effect in muscle strengthening or balance. Moreover beyond drugs there are other interventions often overlooked: supplementation with calcium, exercise programs, orthoses, vitamin D, and fall prevention.

2. Calcium, vitamin D and vitamin D analogues

All of the studies on the effectiveness of anti-osteoporotic drugs required the taking of calcium and vitamin D and recent findings reveal a decreased effectiveness of therapy in individuals with low levels of vitamin D during the therapy (Nieves et al., 1998; Koster et al., 1996; Adami et al., 2009). Trials reporting bone-mineral density, calcium and calcium in combination with vitamin D were associated with a reduced bone loss at the hip and in the spine. A positive treatment effect on bone-mineral density was evident in most studies (Tang et al., 2007). In opposition to findings on the use of calcium and vitamin D together, studies which researched the relative role calcium or vitamin D separately, produced conflicting results. Moreover, calcium and vitamin D or vitamin D by itself increase muscular strength and decrease the number of falls Bischoff-Ferrari, et al., 2006; Bischoff et al., 2003).
Pooled data comparing vitamin D alone with placebo or no treatment showed no statistically significant effect on vertebral fracture or deformity. Vitamin D (including 25-hydroxy vitamin D) with calcium was no more effective than calcium alone on vertebral fracture. Evidence has shown that vitamin D alone was less effective than calcium for the prevention of vertebral fracture or deformity. There was no evidence of a statistically significant preventive effect on clinical vertebral fractures from the administration of vitamin D and calcium and vitamin D plus calcium versus placebo or no treatment. In participants with osteoporosis no statistically significant effect of alfacalcidol (1-alpha-hydroxy vitamin D3) compared with vitamin D and calcium on people with new vertebral deformities was found. Calcitriol (1,25 dihydroxy vitamin D3) and additional supplementation with calcitriol in people with osteoporosis already taking calcium had no statistically significant effect on new vertebral deformity. No statistically significant effect on the number of people developing new vertebral deformities receiving calcitriol plus vitamin D and calcium versus vitamin D and calcium was found. Overall, there was no statistically significant effect on the incidence of vertebral deformities with calcitriol versus calcium. When calcitriol was compared with vitamin D in people with pre-existing osteoporosis no statistically significant effect was seen for vertebral deformities (Avenell et al., 2009).

3. Exercise in osteoporosis

3.1 Biomechanics and mechanobiology of bone

One useful introduction to understanding the response of bone in physical activity is to understand bone's morphology and mechanical properties (Dionyssiotis, 2008b). Bone is a unique material, within the functional structure of the skeleton, which is strong enough to withstand the demands of intense physical activity and external forces exerted, adjusted to changes in those requirements, and light in weight to allow efficient movement and energy saving. The mechanical capacity of bone is a function of internal properties of material (mass, density, stiffness, strength) and geometrical characteristics (size, shape, thickness of cortical cross-sectional area and architecture of trabeculae). The peripheral bone adapts to mechanical loading through endosteal resorption and periosteal apposition of bone tissue

(Figure 1) (van der Meulen, 1993). This increases the diameter of the bone and therefore provides greater resistance to the loads. This adaptive process allows the bone to resist in compression, tension and shear forces, but also to be light enough for efficient and economical movement (Wolff,1870).

Fig. 1. An increase in bone dimensions during development and the gradual age-related endosteal resorption, periosteal apposition and cortical thinning. The increase in bone strength because of the increase in diameter replaces the loss of density (adapted, modified and translated with permission from Dionyssiotis, 2008b).

According to Wolff's law bone will optimize its structure, to withstand the functional burden and to ensure the metabolic efficiency of movement (Wolff, 1892). The loading of the skeleton is described as a strain that produces the modified response of bone to loading. It has been suggested that the osteocyte reacts-perceives the strain and transmits signals to osteoblasts to build bone. The magnitude of strain can be defined as the amount of relative change in length of the bone under mechanical loading (Beck et al., 2001). Mechanical stimulation generated by exercising has at least two opposite effects on bone. The bone as a material is weakened by repeated strains, causing minor damages on bone structure; on the other side, stress strain which exceeds a certain threshold leads to generation and thereby adjusts the strength of the bone load usually applied (Wolff, 1870). This is a feedback cycle, which is usually called as the mechanostat (Frost, 1987a).
The mechanostat theory describes a system in which a minimum effective strain (MES) is essential for maintaining bone (Frost, 1987b). In the overload zone of the system (2000–3000 micro strain) bone is stimulated and new bone is added in response to mechanical requirement. This leads to increased bone strength. Finally, in the pathological overload zone (>4000 micro strain), a minor damage of bone is present and bone mineral is added as part of the repair process. A sufficient number of studies suggest the ability of estrogen to alter the set point of bone strain in responses to mechanical loading as the result of indirect effect of oestrogen' receptors number (Lanyon & Skerry, 2001; Lee & Lanyon, 2004). The decrease in sensitivity of oestrogen receptors as a result of oestrogen deficiency may reduce the response of bone to mechanical loading (Cheng et al., 2002; Jessop et al., 1995). Strain of about 1.000 micro strain increases bone formation, in the presence but not in the absence of oestrogen. Loading forces in the skeleton are caused by gravity (weight bearing), muscles and other external factors.

3.2 Targeted exercise for osteoporosis

Physical activity targeting muscles and balance is the cornerstone of each rehabilitation program for osteoporosis and fracture prevention. Although, in postmenopausal individuals, results of physical activity studies on the positive association of physical activity with bone status are conflicting (Burger et al., 1998; Nguyen et al., 1998). However, it is clear that physical activity is vital in adults (Kelley, 1998; Beitz & Doren, 2004) because it reduces the rate of bone loss during the peri-menopausal period, and decelerates bone loss associated with aging (Asikainen et al., 2004).

In the design of an exercise program to increase bone mass we need to take in mind the following five principles (Drinkwater, 1994) 1. Specificity: The program must be designed to load specific bones or body regions, 2. Overload: To induce stimulation for increasing bone density according to mechanostat theory exercise must overload the bone, 3. Reversibility: In adults, any gains in bone density during an exercise program will be lost if the program stops. However, in children and adolescents the benefits achieved by increased mechanical loading during exercise program remain even if the exercise program stops, 4. Initial Values: The response of bone to increased loading is greater when bone mass is below average. Patients with bone mass below normal will experience greater gains in bone density with exercise programmes, compared with people who have a good bone density, 5. Diminishing Returns: The greatest gains in bone density will be seen early in an exercise program. After the initial increase, the benefits continue but at a slower pace. We added the 6th principle of Variety which is a component of success in all exercise programs. We need to enrich the programs with various exercises and not perform the same exercises, at the same duration and interval. By changing the way of bone and muscle stimulation we challenge them in new way shifting the loading stress causing new results (Dionyssiotis, 2008b).
To summarize the principles: Not all types of physical activities that provide bone loading to the skeleton produce bone mass benefits. Some activities (i.e. a progressive jogging program) charge and stimulate adaptation of the cardiovascular system, but do not stimulate an adaptive bone response that would increase bone density (Khan et al., 2001). The bone has a lazy zone! Each exercise that stimulates the metabolism in the body (i.e. exercise for the cardiovascular system etc.), is not able to stimulate the adaptation of bone to increase bone density. The load on a bone during the exercise should be substantially greater than the load experiencing of the bone during activities of daily living. There is definitely a threshold load which must be reached to generate gains in bone mass. Moreover, loading of the bone should be done in such a way that mimics the physical loads (Skerry, 1997).
There are also activities that provide bone loading at one site of the body, but not at other sites. The osteogenic effects of exercise should be specific to the anatomical sites where the mechanical strain occurs (Lohman et al., 1995). The most common types of physical activities (e.g., gardening, swimming) use many muscles but do not involve targeted bone loading, and therefore do not produce loads heavy enough to exceed the load threshold on bones achieved by usual daily activities (Beck & Snow, 2003; Madalozzo & Snow, 2000). The duration of the physical activity is also important; up to 2 hours per week is considered to positively affect bone mass maintenance (Snow-Harter & Marcus, 1991). Muscle strengthening, weight bearing combined with flexibility, posture control, balance, coordination and training in daily living activities to improve functional capabilities of the subjects should be part of a rehabilitation program in osteoporosis. The following subchapters explain basic exercises of each category (except balance and coordination exercises which will be analyzed in the subchapter of falls prevention) in detail.

3.2.1 Muscle strengthening exercises

In osteoporosis we do not recommend muscle strengthening in generally. Programs are focused on specific regions of the skeleton where fractures are most commonly expected, namely the spine, the hip and the wrist. For this reason in all ages, but particularly in postmenopausal women, exercise programs focusing on muscles in these regions (Table 1) including exercises for the back muscles, the hip and the hand (with weights or pulleys) but also for the thighs because research has shown that the quadriceps is an important muscle for balance and falls prevention.

Type Muscle strengthening	Target	Intensity Frequency Duration	Time to target	Contradictions
Using body weight, free weights, elastic bands, sophisticated equipment in the gym etc.	Increasing strength, stimulate bone to increase bone density (targets are mostly hip muscles, back muscles, biceps, triceps)	8-10 repetitions 2 sets 2-3 times weekly 20-30 minutes	6 months for bone mineral density changes	Subjects with kyphosis should avoid bending and turning the spine and perform the exercises seated

Table 1. Muscle strengthening exercises in osteoporosis; the table summarises the following characteristics of this type of exercise: how we can do them, which are the targets, the intensity, frequency and duration of the program, when to expect the results and the contradictions (Dionyssiotis Y. , 2010 c).

Fig. 2. *(1 to 6) Back muscles:* This group of muscles is usually underestimated in exercise programs, but it requires special attention. The subject should begin warm up in the prone position with the hands flat on the ground and the elbows facing outwards and hold for one minute (photo 1), then raise the head keeping this position for five seconds (photo 2), then return to the starting position. The exercise needs to be repeated five times. The simplest style is photo 3: from the prone position to raise only the hands, with the elbows bent at 90 degrees, whereas it becomes more difficult when the arms are placed at the side of the body and the head is gently raised (photos 4, 5). The exercise needs 15 repetitions 6 times per day (3 in the morning – 3 in the evening). As strength increases it is possible to do more difficult exercises; from a kneeling position, extend one arm and raise the opposite leg. This exercise should be repeated ten times every day. (Dionyssiotis Y., 2010 c).

Fig. 3. *Resistance against a wall (such as push-ups)*: The subject stands opposite a wall and place the hands against the wall with the palms flat on the wall. The feet are spread 15 cm apart. In the next step the subject presses against the wall with the elbows bent and then returns to the initial position. This exercise needs 20 repetitions 3 times per day (Dionyssiotis Y., 2010 c).

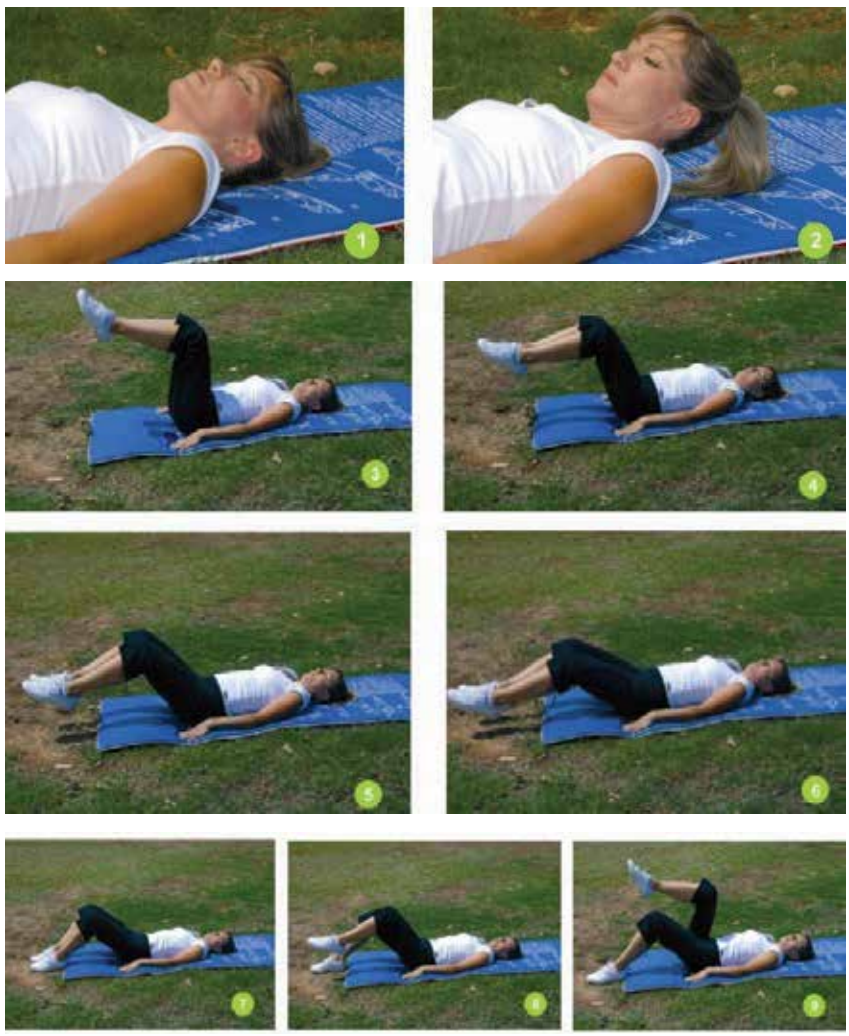

Fig. 4. *Abdominal muscles strengthening*: The subject should begin warm up in the supine position, bringing the chin to the chest for five to ten repetitions (photos 1, 2). The safest exercise for abdominal muscle strengthening includes performing from the supine position with the back flat on the ground, the legs raised and the knees bent at ninety degrees (photo 3). The knees are then extended while lowering the legs with movement coming from the hip joint (photos 4, 5, 6). The spine must be flat on the ground while this exercise is performed. If it is not possible to perform the total movement of this exercise, it should be performed in the half of range as shown in photograph 4. If this exercise causes pain, the subject should alter it as follows: with the legs bent and the sole of the foot on the ground, bend one leg to the abdominals then lower the leg to the ground and the same movement with the other leg (photos 7, 8, 9). Another option is to raise the head with the arms extended to touch the knees (photo 10), (Dionyssiotis Y., 2010 c).

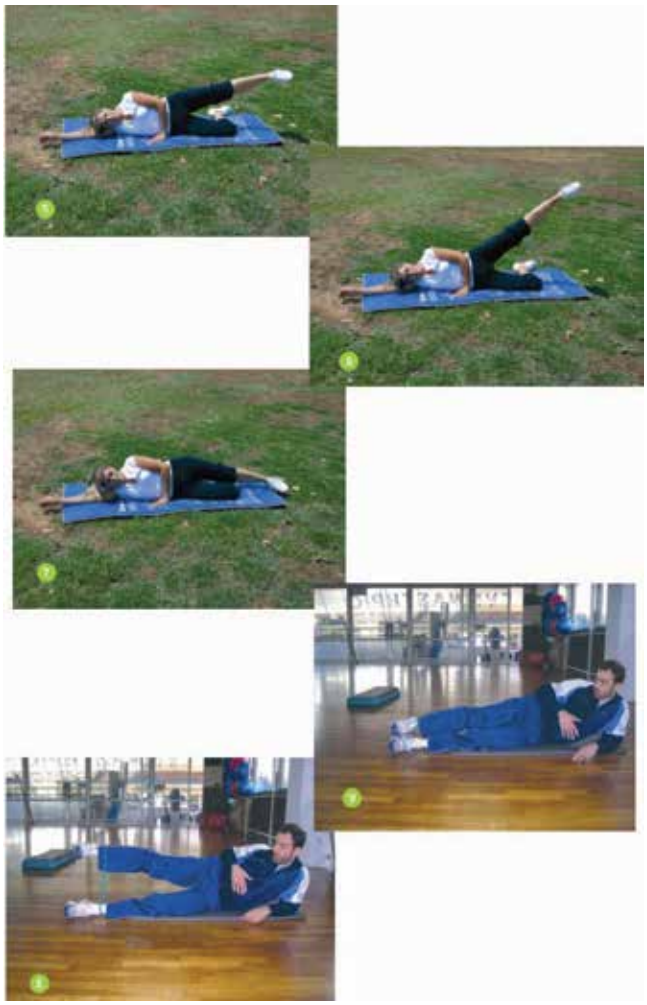

Fig. 5. *Extensor and abductor muscles of the hips:* The subject places the hand on a fixed spot for safety (i.e: chair photo 1) and lift one leg backwards in order to exercise the gluteus maximus muscle extensor muscle (photo 2). Then the movement is repeated with the other leg. From the same position lifts one leg to the side, in order to strengthen the gluteus medius muscle abductor muscle (photo 3); then the other leg follows. For each leg three sets of 15 repetitions are needed. Both exercises can be done with pulleys (at home or at the gym), lying sideways on the ground and also with specific equipment at the gym under the guidance of a qualified instructor (see photos 5-9). Keeping good technique during this exercise is very important and four sets of fifteen repetitions are necessary (Dionyssiotis Y., 2010 c).

Fig. 6. *Quadriceps:* The subject sits upright in a chair with the back as straight as possible, the knees bent and the feet flat on the ground (a chair with armrest is recommended), grips the chair firmly and extends one knee at a time keeping it extended for 4 seconds. This exercise is particularly indicated for elderly people and after hip surgery. The exercise can be done with pulleys and with special equipment in the gym. Each leg needs fifteen repetitions (Dionyssiotis Y., 2010 c).

Fig. 7. *Exercises with dumbbells for the arms:* Exercises for strengthening the biceps and triceps can be done from a standing or seated position. From a standing position the subject flexes the knees slightly, and using medium weights performs three sets of 10 repetitions with each arm (photos 1, 2, 3). Weights can be replaced with pulleys for lower resistance (Dionyssiotis Y., 2010 c).

Subjects need to perform the exercise as above with the same number of repetitions remembering to maintain the correct posture while exercising. For additional safety, exercises should be performed in a seated position by patients with severe osteoporosis.

3.2.2 Weight bearing exercises

Weight bearing exercises are exercises during which the weight of the body passes through the bones. Examples of these types of exercises are walking, jogging, dancing, gardening, tennis, football, basketball and trampoline etc. There is a variety of this type of exercise to suit every age group. The impact to the bone during this exercise should be higher than that during normal everyday activities. Many women believe that housework and the level of

activity it involves constitutes a good level of exercise. However, this is not correct as in order for exercises to be effective they need to be performed with specific technique and systematically.

Type Weight bearing	Target	Intensity requency Duration	Time to target	Contradictions
Walking, jogging, dancing, gardening, tennis, basketball etc.	Maintenance of bone mass Improvement in physical function	40-70% max. Power 3-5 times/ week 20-30 min	9-12 months to improve BMD	Bending and turning in patients with osteopenia

Table 2. Weight bearing exercises; the table summarises the following characteristics of this type of exercise: how we can do them, which are the targets, the intensity, frequency and duration of the program, when to expect the results and the contradictions (Dionyssiotis Y., 2010 c)

Fig. 8. *Walking:* Dynamic walking is the best option for prevention of osteoporosis. Simple walking is not enough; it should be in an open environment without obstacles, not around the house or workplace. Dynamic walking differs from regular walking and to achieve maximum benefit to the skeleton a special technique is required. Brisk walking (dynamic walking) does not require any special equipment except for a good pair of training shoes. Moreover it has the advantage of low risk of injury. Walking should begin at a normal pace, progressively increasing after five minutes to a medium and then to a fast pace for twenty minutes. The pace must be sufficient to allow normal speech but not so fast that the person is out of breath. The level of intensity however should be sufficient for the person to sweat. In order to move the feet faster it is necessary to move the hands faster. Arms should move in the opposite direction to the feet. During the movement of the hands, the subject need to flex the elbows and keep the arms close to the body. Attention should be paid to change of pace, using bigger steps and the feet should be kept in a forward facing direction and not sideway. This kind of walking should be done as often as possible (Dionyssiotis Y., 2010 c).

Fig. 9. *Dancing* as exercise is safe and social which in turn makes this an attractive activity. Jumps and aerobic weight bearing exercises during dancing or gymnastics are related to increasing and maintaining bone density. Traditional Greek dances include movement like jumps, sideways steps and squatting which have a weight bearing effect on the hip and spine (Dionyssiotis Y., 2010 c).

3.2.3 Postural exercises

The aim of these exercises is to: Eliminate the bent-over position (hunchback), which increases the pressure on the front part of the vertebrae and to improve stability. Exercises can be done in the sitting or standing position with eyes open or closed. The optimal is to be performed in front of a mirror and next to a wall. The reason why the exercises are performed in front of a mirror is that the trainees can see their reflection and can correct the possible mistakes in their posture, with the guidance of the experts (visual biofeedback). The wall assists the safe performance of the exercises.

Fig. 10. *Exercises in patients with osteoporosis for correction of posture: Decompression of the spine*: the exercise starts lying on the ground with the knees bent, the feet flat on the ground, the elbow bent and the palms facing upwards and this position is kept for five minutes. This exercise decompresses the spine and relieves back pain. *Shoulder press:* beginning from the same position, the shoulders are pressed to the ground holding for three seconds, and then the subject relaxes himself and repeats three times. This exercise strengthens the muscles of the upper back. *Leg press:* beginning with the position of exercise 1) above, the subjects extends one leg with foot pointing upwards, presses the full length of the leg into the ground, concentrates for 2-3 seconds and relaxes himself. The same steps are performed with the other leg (4 repetitions with each leg). This exercise helps with posture and strengthens the extensor muscles of the thigh (Dionyssiotis Y., 2010 c).

3.2.4 Flexibility exercises

During aging the body becomes more rigid which results in movement difficulties leading to falls and increasing risk of fracture. For this reason it is necessary to perform exercises to maintain flexibility. The exercises in this category help to maintain the elasticity and the length of the muscle, the range of movement of the joints, improve posture and reduce pain (mostly back pain etc).

Fig. 11. *Stretching the pectoralis major (stretching of the chest):* From the standing or sitting position (for greater safety), with the arms bent at the elbows and to the side of the torso, the subject moves the elbows backwards (photo 1). The arms can also be raised in front of the chest with the elbows bent up to the height of the shoulders (photo 2) and then spreads open the arms stretching them out (photo 3). The exercise should be performed daily with 10 repetitions, 3 times (Dionyssiotis Y., 2010 c).

Fig. 12. *Stretching the upper torso:* In this exercise the subject stands or sits on a comfortable chair, the fingers are placed behind ears, palms facing forwards and elbows pointing outwards (photo 1). Stretching the chest by pushing the elbows backwards (without pressing the head) is followed holding this position for 4 seconds and then bringing the elbows together, in front of the face, in order to stretch the muscles of the upper back (photo 2). Exercise is repeated 5-10 times (Dionyssiotis Y., 2010 c).

Fig. 13. *Stretching muscles of the lumbar spine:* The subject is kneeling on the floor with knees slightly apart (photo 1), raises the arms high towards the ceiling and carefully bends forwards, until the palms touch the floor (photo 2, 3), keeping this position for several seconds and repeats 5 times (Dionyssiotis Y., 2010c).

3.2.5 Exercises to improve functional ability – Osteoporosis and daily living activities

The program of exercises becomes more efficient if combined with the use of proper body mechanics and posture in everyday activities.

Fig. 14. *Lifting, carrying and placing weights; the correct and wrong way to lift and place objects:* The correct way for the osteoporotic patient to lift an object is to bend the knees, the hips and the ankles so that the object is at waist level. Bringing the object towards him with both hands and returning to the upright position using the strength of both feet. The spine should be straight during this movement, keeping the head and chest upright and the abdominal muscles tight. An osteoporotic patient is not allowed to lift more than 5-10 kg. The subject stands next to the object keeping the back straight bending the knees and lifting the weight using the strength of the feet and not that of the back, avoiding turning or rotating during the weight lift. The weight must be kept at the level of the waist. When transferring a heavy object, it is preferable to push rather than to pull it and while carrying a weight to separate it evenly on both sides of the body. The abdominal muscles should be flexed, so that the back is in the correct position (Dionyssiotis Y., 2010 c).

Fig. 15. *The correct way for the osteoporotic patient to get up from chair:* The head and the chest must be in the upright position, the body must be bent forward using the hip joint and the base of the spine must be slightly bent with the help of abdominal muscle contraction. Standing up is achieved using the leg muscles. The subject should sit at the edge of the chair with feet slightly behind the knees, pushing forward by placing the weight on toes of the feet while getting up. If necessary the arm rests can be helpful in getting up from the chair. With this way subject is getting up keeping the back and the neck straight (Dionyssiotis Y., 2010 c).

3.2.6 Whole body vibration as antiosteoporotic intervention

Vibration platforms are used in rehabilitation of osteoporosis, based on the concept that non-invasive, short-duration, mechanical stimulation could have an impact on osteoporosis risk. The mechanical loading of bone can be done with application of non-physiological factors, such as vibrations that combine dynamic loads and high intensity loading on the skeleton (Dionyssiotis, 2008b). The implementation should be shortly and has specific indications, contraindications and adverse reactions. These machines cause whole-body vibration. The vibration is a mechanical stimulation of the whole body; the person is standing on the vibration platform trying to keep his head and body straight and upright. All the muscles that keep the body in this position are forced to react to the oscillating movements provided by the device. The duration of this exercise depends on the type of machine in order to have measurable results and benefits.

According to the mechanostat theory bones need great forces for their development. The mechanical loading of bone can be done either with usual exercise activities as those reported in subchapter 3.2 or by applying non-physiological factors, such as body vibration. With platforms goal is achieved safely, without injury and quickly. Mechanical loads are applied in a dynamic way with a high intensity defined by its frequency (hertz) and magnitude, where magnitude is expressed as vertical acceleration (g; 1g=9.8 m/s^2 acceleration due to gravity) or vertical displacement (millimeters). In the scientific world there is a debate about how exercise with vibrations develops bones. One theory holds that low vibration intensity but high frequency can cause osteogenic response by direct action on bone (Rubin et al., 2001). They support the following concept: because of small strains caused by this mechanism, there are benefits to bone without the risk of causing mechanical damage.

The credibility of this theory has been demonstrated in sheep, where one arm vibration caused a 34% increase in volumetric trabecular bone mineral density of the femur (Rubin et al., 2002). Moreover through this type of vibration trabecular bone density of the tibia in children with cerebral palsy was increased, whereas bone loss was expected without treatment (Ward et al., 2004). A recent study demonstrated benefits in postmenopausal women: an increase of 2.2% and 1.7% in bone density of the hip and spine respectively (Rubin et al., 2004). The second theory supports the concept of the important action of the muscles; vibrations make bones stronger through powerful muscular contractions (Rauch & Schoenau, 2001; Rittweger et al., 2000; Schiessl et al., 1998). In postmenopausal women, bone density increased by 1% after 6 months when vibration of static and dynamic knee-extensor exercises on a vibration platform (35-40 Hz, 2.28-5.09g) was performed which also increased muscle strength (Verschueren et al., 2004). However, these increases were also evident in the comparison group of women who performed traditional resistance exercises. A study performed on immobilized young men (Berlin bed rest study) concluded that a combination of vibration and resistance exercises prevent bone loss due to immobilization (Rittweger & Felsenberg, 2004). A systematic review and meta-analysis found significant but small improvements in BMD in postmenopausal women and children and adolescents, but not in young adults (Slatkovska et al., 2010).

Fig. 16. Galileo vibration platform (Novotec Medical GmbH, Pforzheim, Germany, with permission).

3.3 Exercise and bone density

The effect of aerobic exercise on bone density has been studied by review papers which report a decrease in bone loss at the spine and wrist but not at the hip (Bonaiuti et al., 2002; Martyn-St James & Carroll, 2008; Martyn-St James & Carroll, 2006). In meta-analysis studies which reviewed the effects of walking on bone density showed that walking has a small effect on sustaining bone density at the spine in postmenopausal women, however it has a significant positive effect on the femoral neck and concludes that other types of exercises which provide larger "targeted" weight bearing forces are needed to maintain bone density in this group (Martyn-St James & Carroll, 2006). In a review of 35 RCT's it was shown that in premenopausal women and in postmenopausal women intense exercise probably had a

positive effect on the femoral neck and in spinal lumbar bone density, where less intensive exercise also helped (Kerr et al., 1996). In one meta-analysis study it was found that systematic high intensity resistance training is required for the maintenance of spinal lumbar bone density in postmenopausal women; however weight bearing exercise is necessary to help bone density of the hip beyond any other therapeutic intervention (Kelley, 1998).
In a three year period during the EFOPS study (Erlangen Fitness Osteoporosis Study), which included a exercise protocol with a combined strengthening program, jumping and high intensity resistance training in early onset postmenopausal women, sustained the bone density in the spine, the hip and in the heel, however not in the forearm. A well planned study which compared muscle strengthening exercises with weights and with resistance exercises with repetitions showed that the weight used was more important than the number of repetitions in postmenopausal bone (Engelke et al., 2006). A similar analysis in men revealed similar results (Kelley et al., 2000). With respect to bone quality a review study which used peripheral quantitative computed tomography (pQCT) revealed that exercise possibly increased bone mass and geometry in postmenopausal women, changes which theoretically increase bone resistance. Specifically, the effects of exercise are moderate, area specific and act primarily on cortical rather than trabecular bone (Hamilton et al., 2010).

3.4 Combined exercise with calcium, bisphosphonates

A decreased rate of bone loss in postmenopausal women undergoing exercise and taking calcium supplements is reported in comparison with exercisers only suggesting that calcium deficiency reduces the efficacy of loading to improve bone mass (Prince et al., 2006). In another study included 1890 pre- and postmenopausal women measured by quantitative ultrasound (QUS) at the heel and assessed with validated questionnaire according to physical activity and daily calcium consumption (greater than or less than 800 mg/day) was found that systematically active premenopausal and postmenopausal women had significantly higher values of QUS parameters than their sedentary and moderately active counterparts. Moreover a statistically significant difference in QUS T-score between sedentary premenopausal women and those who exercise systematically was found suggesting that vigorous physical activity is a regulator of bone status during premenopausal years (Dionyssiotis et al, 2010a).
In a randomized, double-blind, placebo-controlled trial the primary endpoint was the 12-month change in bone mass and geometry of the effects of weight-bearing jumping exercise conducted in an average 1.6 ± 0.9 (mean ± SD) times a week and oral alendronate, alone or in combination, measured with dual-energy X-ray absorptiometry and peripheral computed tomography at several axial and limb sites. A total of 164 healthy, sedentary, early postmenopausal women were randomly assigned to one of four experimental groups:(1) 5 mg of alendronate daily plus progressive jumping exercise, (2) 5 mg alendronate, (3) placebo plus progressive jumping exercise, or (4) placebo. Alendronate daily was effective in increasing bone mass at the lumbar spine and femoral neck but did not affect other bone sites. Exercise alone had no effect on bone mass at the lumbar spine or femoral neck; it had neither an additive nor an interactive effect with alendronate at these bone sites. However, at the distal tibia the mean increase in the section modulus (a bone strength parameter) and in the ratio of cortical bone to total bone area were statistically significant in the exercise group compared to the non exercise group, indicating exercise-induced thickening of the bone cortex. The authors concluded that alendronate is effective in increasing bone mass at

the lumbar spine and femoral neck, while exercise is effective in increasing the mechanical properties of bone at some of the most loaded bone sites (Uusi-Rasi et al., 2003).
On the other hand the combined and separate effects of exercise training and bisphosphonate (etidronate) therapy on bone mineral in postmenopausal women were investigated in forty-eight postmenopausal women randomly assigned to groups that took intermittent cyclical etidronate; performed strength training (3 d/week) and received matched placebo; combined strength training with etidronate; or took placebo and served as non-exercising controls. Bone mineral was assessed by dual-energy X-ray absorptiometry before and after 12 months of intervention changes in bone mineral density (BMD) of the lumbar spine were greater in the subjects given etidronate compared with placebo, while exercise had no effect. No effect of etidronate or exercise on the proximal femur and there was no interaction between exercise and etidronate at any bone site was found (Chilibeck et al., 2002).

4. Modern orthoses in osteoporosis

Traditionally, spinal orthoses have been used in the management of thoracolumbar injuries treated with or without surgical stabilization. The vast majority of orthoses, however, are used in patients with low back pain (Perry, 1970). These orthoses, however, have never been tested under standardized conditions. Especially, no prospective, randomized, and controlled clinical trials are available to document efficacy according to the criteria of evidence-based medicine. Moreover, there is a lack of specific studies comparing various types of braces and orthoses. This is also the case for osteoporosis, in which approximately one-fourth of women above 50 years of age have one or more vertebral fractures (Melton, 1993).
Even though, it is widely accepted that spinal orthoses whether made of cloth, metal, or plastic, or whether rigid or flexible, relieve pain and promote the healing process by stabilizing the spine i.e. reducing the load applied on the anterior column and vertebral body by restraining any attempt of forward flexion. The most broadly used types of spinal orthoses use a three-point pressure system (Dionyssiotis et al., 2008; Mazanec et al., 2003): a) the TLSO type (Knight-Taylor, Jewett, CASH or Cruciform Anterior Sternal Hyperextension brace, Boston); that provides support to the thoracolumbosacral spine by making it adopt an anatomically correct position. The CASH or Jewett brace has been favoured for patients with acute vertebral fractures. The goal of these braces is to provide forces to encourage hyperextension. However, a drawback to these orthoses is the limited compliance because of their rigid configuration, b) the PTS (Posture Training Support) type, or the newer postural training support vest with weights (PTSW), two orthoses made of a softer material, gained popularity because of their improved comfort and increased compliance. The postural training support is worn over the shoulders similar to a mini-backpack and has a pocket into which small weights (total 1.75 lb) weights are added. The postural training support vest with weights is similar except that it is fashioned as a vest, with a Velcro attachment that fastens around the abdomen (Sinaki & Lynn, 2002), c) Spinomed and Spinomed active based on biofeedback theory (Pfeifer et al., 2004; Pfeifer et al., 2011); Spinomed consists of an abdominal pad, splint along the spine, back pad, and a system of belts with Velcro. The back orthosis consists of a back pad, which is workable as a cold material, and a system of belts with Velcro. This allows adjustments for individual sizes by an orthopedic technician. The orthosis weighs 450 g and is worn like a back pad and d) Osteomed, which is based upon

the gate control theory of pain (Vogt et al., 2008); the external appearance of the orthosis Osteomed resembles an item of clothing characterised by a constructively functional cut with Velcro tabs exerting pressure in the lumbosacral region as well as air chamber pads fixed in the paravertebral and lumbosacral areas which are filled with air to between 2/3 and ¾ of their maximum capacity (Vogt et al., 2008).

Fig. 17. Front, back and lateral view of the Spinomed (unpublished images of Dionyssiotis et al.)

Fig. 18. Front, back and lateral view of the Spinomed active orthosis for men and women (Medi-Bayreuth, Bayreuth, Germany, with permission).

In a controlled pilot study with a 4-week observation period the strength of the back extensors was reduced to below the initial value in 40% of female patients wearing a stable orthotic device pointed out that orthotic devices impose a risk of reduction in muscular strength (Kaplan et al., 1996). On the contrary, recently published results of women with established osteoporosis and/or an angle of kyphosis more than 55 degrees wearing Spinomed for at least 2 hours/day for 6 months showing significantly decreased back pain ($p=0.001$) (evaluation was performed using visual analogue scale at the beginning and 6 months follow up of the examination) and increased personal isometric trunk muscle strength (figure 19) (Dionyssiotis et al., 2010b). Moreover in another Spinomed study subjects separated in two groups, the control and orthosis group, who switched after 6 months. Wearing the orthosis resulted in a 73% increase in back extensor strength, a 58% increase in abdominal flexor strength, most likely because of increased muscular activity while wearing the orthosis, a 11% decrease in angle of kyphosis, a 25% decrease in body sway, a 7% increase in vital capacity, a 38% decrease in average pain, a 15% increase in well-being, and a 27% decrease in limitations of daily living (Pfeifer et al., 2004).

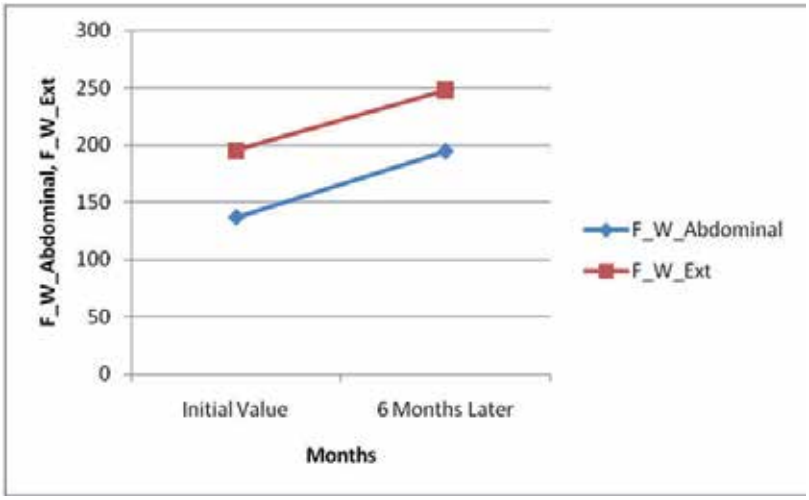

Fig. 19. Schematic presentation of differences in the values of personal isometric force: Force (F)/Weight (W) in abdominals and extensors muscles (F/W abdominals and F/W extensors, respectively, after 6 months wearing Spinomed orthosis (F: force in Newton, W: weight in Kg), measured with ISO-RACK device (Digimax, MechaTronic, Hamm, Germany). Figure adapted from Dionyssiotis et al., 2010b (with permission).

According to the results obtained from Osteomed studies, the orthosis brings an active erection of the spine of 60% on average of the deliberate maximum possible active erection. The wearing of the orthosis leads to an improvement of posture and statics (Vogt et al., 2005), a straightening of the spine of on average 46% of the conscious maximum achievable straightening (Vogt et al., 2008) and a statistically significant and clinically relevant reduction in chronic back pain by approximately 25% in female patients with osteoporosis worn it in a period of 2.5 months (Fink et al., 2007).

Fig. 20. a) Front view of the Osteomed osteoporosis orthosis (Osteomed, Thaemert Ltd, Germany), b) Dorsal view of the Osteomed osteoporosis orthosis (for demonstration purposes the air chamber pads are shown on the outside), c) View of the orthosis without air chamber pads, d) View of the placebo device (adapted from Fink et al., 2006, with permission).

Strengthening the back muscles not only maintains bone density in the spine but also reduces the risk of vertebral fractures. Ten years after a 2-year back exercise program in women fractures, both wedging and vertebral compression fractures, were significantly less (only 11% in the exercise group as compared to 30% in the control group) several years after the exercises were discontinued (Sinaki et al., 2002).

5. Prevention of falls and fall related fractures

An important issue in rehabilitation medicine is the prevention of falls and fall related fractures. Falls is a serious problem facing elderly persons. Falling results in increased mortality, morbidity, reduced functioning and premature nursing home admissions. Falls generally result from an interaction of multiple and diverse risk factors and situations, many of which can be corrected (Dionyssiotis et al., 2008a).

Falls can also result in deterioration of physical functioning and quality of life due to injury or due to fear of falling; 16% of fallers reported that they limited their usual activity because of fear of falling and one third of fallers reduced their participation in social activities (Nevitt et al., 1991). Fear of falling is reported by one in four older people in the community and can lead to distress and reduced quality of life, increased medication use and activity restriction, further decline in physical functioning, greater falling risk and admission to institutional care (Yardley et al., 2005). It is necessary to assess possible intrinsic and extrinsic risk factors for falls, as well as the exposure to individual's risk (Todd & Skelton, 2004).

Identifying risk factors is as important as appreciating the interaction and probable synergism between multiple risk factors because the percentage of persons falling increased from 27% for those with no or one risk factor to 78% for those with four or more risk factors (Tinetti et al., 1988). Important potentially modifiable risk factors for community-dwelling older adults are: mental status and psychotropic drugs, multiple drugs, environmental hazards, vision, lower extremity impairments, balance, gait status and for institution-dwelling older adults: mental status, depression, urinary incontinence, hypotension, hearing, balance, gait, lower extremity impairments, low activity level (exercise less than once a week), psychotropic drugs, cardiac drugs, analgesics and use of a mechanical restraint; non-modifiable risk factors (i.e. hemiplegia, blindness) also exist (Moreland et al., 2003).

Interventions to prevent falls may be planned to reduce a single internal or external risk factor of falling or be broadly focused to reduce multiple risk factors simultaneously (Sjösten et al., 2007). Single evidence based interventions include exercise, reassessment of medications and environmental modification (American Geriatrics Society [AGS], British Geriatrics Society [BGS], and American Academy of Orthopaedic Surgeons [AAOS], 2001; Tinetti, 2003). Although exercise has many proven benefits, the optimal type, duration and intensity of exercise for falls prevention remain unclear. Older people who have had recurrent falls should be offered long-term exercise and balance training (Dionyssiotis et al., 2008a).

5.1 Exercise for falls prevention

5.1.1 Balance exercises

Without good balance, there is always the danger of fracture. This type of exercise is the most important in falls prevention. Simple exercises for balance are walking heel to toe beside a wall or rail and balancing on one foot. The purpose of the exercises is the development of synchronized movements, resulting in balanced sitting and standing positions (Dionyssiotis, 2010c).

Fig. 21. Heel to toe exercise and balance standing on one foot: walking heel to toe beside a wall or rail for a short time. In alternative standing at the side of a chair (for safety) and leaning on the chair with one hand, whereas at the same time the opposite leg is raised with the knee bent as shown in the picture. Subjects perform the exercise, first with open and then with closed eyes and continue by changing side and leg of support. Ten repetitions for each leg are necessary (Dionyssiotis Y., 2010 c).

5.1.2 Coordination exercises

These exercises help the cooperation of muscle and nerves in order to avoid falls and fractures and should be done routinely every day for at least 5 minutes. This category includes exercises such as marching, walking around a chair and throwing and catching a ball.

Fig. 22. Marching (photo 1) and walking around a chair (photos 2 and 3). Marching is an excellent exercise for coordination. Training consists of the simultaneous movement of one arm and the opposite leg in turn. During the execution of the exercise, the head must look forward; the arms must be slightly bent on the elbows and must reach up to the height of the shoulders. Placing a chair in a room, to make it able to walk around it on all sides, walking clockwise and then counter clockwise, as fast as they can, (should stop before getting dizzy) and repeat for 5 times (Dionyssiotis Y., 2010 c).

Fig. 23. Exercise balls. Throwing and catching a ball is a very good exercise for coordination. The exercise is performed for security, from the sitting position and the ball thrown at a low height (photos 4 and 5). After enough practice at the previous exercise and while still in the sitting position, the ball can be thrown to and from another person sitting opposite (photos 6 and 7), (Dionyssiotis Y., 2010 c).

Type Balance & Coordination	Target	Intensity Frequency Duration	Time to target
Execution in parallel bars or next to a wall or a chair, because it has to be safe, in order to avoid falls	Improving coordinated movements resulting an improve in balance in seated and standing position	medium intensity 5-7 times/week 5-10 min	2-4 weeks

Table 3. Balance and coordination exercises are important for falls prevention; the table summarises the following characteristics of this type of exercise: how we can do them, which are the targets, the intensity, frequency and duration of the program and when to expect the results (Dionyssiotis Y., 2010 c).

5.1.3 Tai Chi

Tai Chi is a promising type of balance exercise, although it requires further evaluation before it can be recommended as the preferred method for balance training (AGS, BGS, AAOS, 2001). Tai Chi which consists of slow, rhythmic movements emphasizing on the trunk rotation, weight shifting, coordination, and a gradual narrowing of the lower extremities position is thought to be an excellent choice of exercise for the elderly.There is experimental evidence from both cross-sectional and longitudinal studies that Tai Chi exercise has beneficial effects on balance control and that the postural stability is improved

more by Tai Chi than by other types of exercise (Graafmans et al., 1996). Although Tai Chi is probably the exercise programme we would least recommend to people who have previously suffered fractures because they show a level of frailty that means they could not fully participate in Tai Chi unless it was adapted so much it was no longer dynamic balance training (Skelton D, personal communication). From the most training studies after hip fracture it seems that combined training with task-specific and functionally based exercises may be a sensible way of retraining leg strength, balance and gait ability in elderly people after a hip fracture. The training thus may include a variety of gait exercises, step exercises, stair climbing, and rising from and sitting down on a chair (Sherrington et al., 2004; Hauer et al., 2002; Lindelφf et al., 2002).

5.1.4 Clinical trials and multifactorial intervention

A review about the effectiveness of interventions to prevent falls in older adults concluded that exercise programs help prevent falls with no differences between types of exercise (Chang et al., 2004).The results from the FICSIT trials (Frailty and Injuries: Cooperative Studies of Intervention Techniques) suggest that interventions that addressed strength alone did not reduce falls. On the other side balance training may be more effective in lowering falls risk than the other exercise components (Lord et al., 2007).

Others concluded that exercise programmes must be regular and sustainable to be effective but more trials are required to determine the exercise type, frequency, duration, and intensity that are most effective in lowering falls risk in different groups of older people (Gardner et al., 2000). However, as ageing is related with reduced physical functioning, exercise prescription for falls prevention, beyond balance and strength training, may include exercises to increase the functional capabilities in all elderly. The suggested guidelines especially for the Greek population are low intensity balance exercises (tandem walking and standing on one's foot) combined with coordination exercises. Individuals who are frail, severely kyphotic or suffer from pain or poor balance may benefit from water exercise (hydrotherapy). People are also advised to undergo strengthening exercises of the quadriceps, hip abductors/extensors, back extensors and the arm muscles (Dionyssiotis et al., 2008a)

Frequent fallers should have their medications reviewed. Studies have indicated that the use of medication is a potential cause for falls (Hartikainen et al., 2007). Central nervous system drugs, especially psychotropics warrant particular attention, since there is very strong evidence that use of these medications is linked to the occurrence of falls. Reducing the total number of medications to four or fewer, if feasible, has also been demonstrated to reduce the risk of falling (AGS, BGS, AAOS, 2001; Tinetti, 2003). Environmental hazards could be a cause of falls (Lord et al., 2007). In reducing environmental hazards, falls prevention programs may need to provide and install safety devices particularly in the homes (Wyman et al., 2007). Studies have shown that when older patients at increased risk of falls are discharged from the hospital, a facilitated environmental home assessment should be considered (AGS, BGS, AAOS, 2001; Tinetti, 2003).

There is emerging clinical evidence that alfacalcidol, a prodrug of D-hormone, improves muscle function (Runge & Schacht, 2005). In community dwelling elderly women and men with a total calcium intake of more than 500 mg daily and normal vitamin D serum levels 1 μg alfacalcidol daily reduced significantly the number of falls (-54%) and fallers (-55%) (Dukas et al., 2004). Other authors reported that cholecalciferol-calcium supplementation

reduces falls by 46% to 65% in community-dwelling older women, but has a neutral effect on falls in men (Bischoff-Ferrari et al., 2006). Prevention may be even more effective when multiple risk factors of falls are taken into account. Most multifactorial fall prevention programmes have been successful in reducing the incidence of falls and risk factors of falling, especially when prevention has been individually tailored and targeted to populations at high risk of falling (Moreland et al., 2003).
Multifactorial interventions should include: a) among community-dwelling older persons (i.e. those living in their own homes), gait training and advice on the appropriate use of assistive devices, review and modification of medication, especially psychotropic medication, exercise programs, with balance training as one of the components, treatment of postural hypotension, modification of environmental hazards and treatment of cardiovascular disorders, b) among older persons in long-term care and assisted living settings staff education programs, gait training and advice on the appropriate use of assistive devices and review and modification of medications, especially psychotropic medications (AGS, BGS, AAOS, 2001; Tinetti, 2003).

6. Rehabilitation of common osteoporotic fractures

Successful operative treatment of hip fracture victims is necessary for the optimization of post-injury mobility and the functional recovery of the patient (Koval, 2005). Two evidence-based clinical practice guidelines suggesting possible treatments and rehabilitation pathways for hip fracture patients, agree that it would be best if they underwent multidisciplinary rehabilitation (Scottish Intercollegiate Guidelines Network [SIGN], 2002; Chilov et al., 2003). Multidisciplinary rehabilitation can be defined as the combined and coordinated use of medical, social, educational and vocational measures for training or retraining the individual to the highest possible level of function (Cameron, 2005).
Hip fracture patients should start breathing exercises so that pulmonary secretions are drained, thus reducing the risk of atelectasies and other complications deriving from the pulmonary system. "Pump like" energetic exercises (ankle pumps) and dorsal/plantar flexion of the foot, knee joint flexion, exercises for the hip and thigh, abduction exercises for the gluteal muscles and exercises for the quadriceps are important. Exercises of the upper extremities and trunk must also be part of the rehabilitation program, so that the patient can move in bed, stand up from a chair and later on be able to mobilize himself by using crutches or a stick. Abdominal and dorsal muscles should also be exercised isometrically and then energetically, in order to minimize the risk of low back pain during weight-bearing exercises (a detailed rehabilitation program is published in Dionyssiotis et al., 2008a).
After a vertebral fracture a program of physical therapy is necessary and helps prevent deformity by strengthening anti-gravity muscles and promoting postural retraining. Breathing exercises promote thoracic expansion and improve the heavily degraded pulmonary function found in patients with spinal osteoporotic fractures (Pfeifer et al., 2004). Instruction on the proper way of lifting things, as well as how to appropriately use a walker or a cane, could be beneficial and thus is strongly recommended. Patients with fractures could perform low-intensity exercise and gentle strengthening programs (e.g., Tai Chi and hydrotherapy) and are strongly recommended to avoid high impact exercise or movements, so that they avoid suffering new vertebral fractures (Tosi et al., 2004). Forward bending of the spine or flexion exercises, especially in combination with twisting, should be avoided.

This includes several old favourite exercises which are now considered outdated, namely straight-leg toe touches and sit ups (or crunches) for strengthening the abdominal muscles (Bassey, 2001). The latter are associated with a dramatically increased rate of vertebral fracture in osteoporotic women (89% compared to 16% of those who did extension exercises) (Sinaki & Mikkelsen, 1984). As the acute fracture pain subsides, a walking program can begin with gentle strengthening exercises focusing on spinal extensor muscles (Bonner et al., 2003). A carefully supervised rehabilitation program should be started after 3 to 4 months, to strengthen the spinal extensor and abdominal muscles more aggressively (a detailed rehabilitation program is published in Dionyssiotis et al., 2008a).

Physical therapy after a Colles' fracture consists of muscle strengthening, motion range recovery, wound healing and scar adhesion. Early reduction of oedema is of primary importance in determining hand functions. Elevation of the hand above the heart's level and an active range of motion exercises are instructed to facilitate the pumping action of hand muscles to decrease swelling. Physical modalities and exercise programs consisting of passive and active range of motion; transverse scar massages, progressive resistive exercise, focusing on strengthening both extrinsic and intrinsic muscle groups of the hand are necessary (Morey & Watson, 1986; Dionyssiotis et al., 2008a). Physical therapy is followed by occupational therapy for 3 weeks (Christensen et al., 2001).

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Physical Exercise for Prevention of Falls and Fractures

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1. Introduction

Osteoporosis is a metabolic bone disease that specially affects postmenopausal women resulting in devastating effects associated to the high social-economic impact in the population in general. The World Health Organization defines osteoporosis as a skeletal disorder characterized by a reduction in bone mass with alterations in the micro-architecture of the bone tissue leading to a decrease in bone resistance and increased susceptibility to fractures (World Health Organization, 1994; Bennell et al., 2000; Gali, 2001; Szejnfeld et al., 2007).

Bone is a highly metabolic active tissue that maintains its remodeling throughout life (Hunter & Sambrook, 2000). On the other side, bone mineral density is a result of a dynamic process of bone formation and resorption called remodeling. Resorption causes the tissue deterioration, while its deposition is responsible for the reconstruction and strengthening of the deteriorated tissue. This process occurs through life in cycles of four to six months (Bemben et al., 2000).

The bone wear out in daily life demands a process of permanent remodeling. This remodeling process renews in a year about 10% of the skeleton, that is, all bone tissue is remade every 10 years (Manolagas, 2000).

The global rate of bone resorption is regulated by the osteoclastic differentiation through the regulation of fundamental functional proteins, which specific role is to control its migration and resorption (Bruzzaniti & Baron, 2006). Osteoblasts are the cells responsible for the bone formation through the synthesis and mineralization of the skeleton and formation of osteoids (Bodine & Komm, 2006). Because the osteoids are not able to reproduce when they are damaged they go through a process of apoptosis, releasing osteoclast-forming inductors which will phagocyte them. This is the first stage for its replacement that will be performed by the osteoblasts (Manolagas, 2000).

In a regular remodeling process, there is a balance between the enzymatic production of osteoclasts and the production of a primary matrix of collagen and fixation of calcium promoted by the osteoblasts (Position Statement, 2002).

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Human beings reach their bone mass peak around age 30 years being strongly affected by genetic, representing 60-80% of the bone mass peak showed by an individual (Ramalho & Castro, 1999).

In the adult, 90% of bone mass is resting, while 10% is in constant activity to revitalize the bone tissue. Neoformation occurs only after the resorption of a damaged bone. In a year, 25% of the trabecular bone and 1% of cortical bone are remodeled by a still unknown mechanism. During growth, the balance of this renewal is positive. In the adulthood, it is even and after age 40 it starts being negative. During the age where this balance is negative, the portion destroyed is not completely remodeled and around 1% of the bone mass is lost annually (Carvalho, 2006).

The decrease in bone mineral density (BMD) with age is considered as a physiological osteopeny, being a universal phenomenon that affects all races and cultures; non-pathological by itself in most of the individuals, but it is the background to development of osteoporosis and consequently, a higher risk of fractures (Ramalho & Castro, 1999). The sequence of this negative renewal throughout the years is responsible for the primary osteoporosis (Carvalho, 2006).

During the age-related bone loss, there is an unbalance in bone remodeling, with an increase of bone resorption compared to formation. In the stage of accelerated postmenopausal bone loss, there is a high rate of bone remodeling, with an increase in the number of osteoclasts that forms a very deep resorption cavity leading to a trabecular perforation. In the slow process of bone loss, osteoclasts build a bone resorption cavity with a normal depth, however the osteoclasts fail in replacing the new bone in a proper way (Yoshinari & Bonfá, 2000).

The incidence of osteoporotic fractures (Figure 1) is strictly related to the individual bone mass that depends on the speed of loss throughout life as well as the amount of bone tissue in the end of puberty and beginning of adulthood. The great variation in bone mass peak is explained not only by hereditary factors but also by gender, race, eating habits, several hormone influence, body composition of lean mass and body fat, intercurrent diseases, chronic use of medications and physical activity (Brandão & Vieira, 1999).

Like any other chronic disease, the ethiology of osteoporosis is multifactorial. Genetic factors contribute approximately with 46% to 62% of bone mineral density (BMD) whereas other causes include lifestyle, diet and physical exercise (Neto et al., 2002).

Osteoporosis is considered a "silent disease" until a fracture occurs. Approximately 1.5 million fractures per year are attributable to this disease. Only in the USA, these fractures result in 500.000 hospitalizations, 800.000 emergency room visits, 2.6 million physician visits. The treatment cost is high. In 2002, 12 billion dollars to 18 billion dollars were spent (Gass & Huges, 2006). In 1998, cost management of osteoporosis fractures in the UK recorded *942 million pounds* per year (Szejnfeld et al., 2007). Because it is considered a "silent" disease, it may progress for decades before being diagnosed. *Osteoporosis* has become one of the major public health problems. Nowadays, the impact of osteoporosis is compared to the impact caused by most important health problems, such as cardiovascular diseases and cancer (Froes et al., 2002).

It exposes the fallers to a high risk of fractures (Johnell et al., 2005; Siris et al., 2006). The first hip fracture is associated to 2.5-fold increased risk of subsequent fracture (Cólon-Emeric et al., 2003) with a high level of morbidity and mortality (Cathleen et al., 2006).

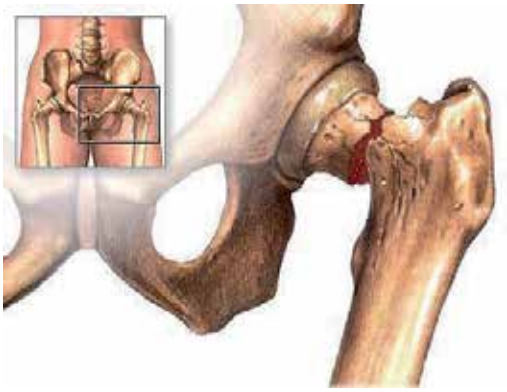

Fig. 1. A femoral neck fracture of the hip.

It is believed that about 25% of menopausal women in the USA will exhibit some kind of fracture as a consequence of osteoporosis. The most severe fractures are the fractures of femur and they are associated with higher medical expenses than all other osteoporotic fractures together (Moreira & Xaxier, 2001). The incidence of these fractures has doubled in the last 25 years and it is estimated that six million people in the world will suffer fracture of the proximal femur in 2050. Fractures resulted from the decrease of bone mineral loss are considered an orthopedic epidemic leading to an increase in costs for several countries and consequently representing a big social and economic problem (Ramalho et al., 2001).
There have been a significant number of evidences showing that the decrease in bone quality, from generation to generation, is caused by a change in life style, having as a main determinant the lack of physical activity. This evidence varies with the biology of the basic bone. However, epidemiological studies indicate that physical activity is the most important factor to maintain bone mass and prevent fractures (Mosekilde, 1995).
Almost all hip fractures (more than 90%) occur as a result of a fall and these fractures are related not only to the decreased bone mass, but also to other factors such as reduction of balance, muscle strength and power in the lower extremities (American College of Sports Medicine [ACSM], 1995; Parkkari et al. 1999). Therefore, aging and alterations in balance and muscle strength, as well as sensorial changes, predispose patients with osteoporosis to a higher risk of having fractures due to falls.

2. Physiology of aging and falls

The aaging process is associated to several anatomic and physiological changes which are directly related to musculoskeletal frailty and falls (Walsh et al., 2006).
The visual system with aging tends to decrease the visual acuity and visual field, also decreasing the speed in adjusting to dark and increase in the *threshold* for *luminosity* (Sloane et al., 1989).
When the somatosensorial system gets old, it show a loss of proprioceptive fibers related to *kinesthetic* sensitivity. Histological studies have shown the decrease in the number of Pacini, Merkel and Meissner corpuscles in the (Sloane et al., 1989).
The main structural and electrophysiological changes in the vestibular system due to aging are: after the age of 40 years, microscopic synaptic changes in the vestibular nerve, increase in the degeneration of the vestibular receptors mainly in the ampullary crest of semicircular

canals and saccule at the age of 50 years, preceding the decrease in the proportion of cells in the Scarpa ganglion. After the age of 60, there is an increase in friction among the fibers of the vestibular nerve, selective loss of density in the myelin fibers leading to a decrease in conduction velocity of the electrical stimuli in the vestibular nerve, decrease of the nystagmic response to caloric and rotational tests in elderly people, decrease in the ptokinetic nystagmus amplitude and pursue eye movements, mainly for the visual stimulus with high speed (Vicini et al., 1989).

Qualitative and quantitative changes in the ciliated cells are observed as well cystic degenerations, fusion of cilia and lipofuscin inclusion in the cell (Isuji et al., 2000).

The loss of ciliated cells is relevant and in general occurs in five sensorial structures of the vestibular system (three semicircular canals, saccule and utricle) in elderly patients, being greater in the crista of semicircular canals than at the saccular and utricular maculae (Isuji et al., 2000).

In the elderly, the vestibulo-ocular reflex (VOR) shows a bigger capacity of compensation than the vestibular-spinal reflex (VSR) aggravating the difficult in maintaining the stability in posture stability (Enrietto et al., 1999; Norré et al., 1987). Another factor that has an influence on the postural instability in the elderly is the alteration in the neuromuscular system.

Studies have shown that the muscle strength reaches its peak around the age of 30 years and it is satisfactory preserved up to the age of 50 years (Deschenes, 2004). However, a decrease in strength is observed around the age of 50 and 60 years, with a faster decrease after the age of 60 years (Krueger et al., 2001). The muscle mass decrease around 50% between 20 and 90 years of age and the number of fibers in the elderly is approximately 20% smaller than in the adults (Rossi & Sadler, 2002).

When measured after the 50s, the progression rate related to a reduction in strength is around 8 to 15% by decade and men, as well as women, show the same pattern of strength decrease during aging (Deschenes, 2004; Krueger et al., 2001). However, longitudinal investigations have shown a greater increase in the strength reduction in seniors than the results found in transversal studies (Deschenes, 2004).

Additional complications in muscle function associated to severe or chronic diseases, hospitalizations after trauma or surgery and lack of activity might accelerate the muscular strength decrease (Krueger et al., 2001). Age-associated decrease of muscle strength mainly results in a substantial reduction in muscle mass that follows the aging process, generating a great loss of muscle mass and an increase in the subcutaneous and intramuscular fat, denominated “sarcopenia” (Wilmore & Costill, 1999; Deschenes, 2004; Hunter et al., 2004; Krueger et al., 2001).

According to Deschenes, 2004, the decrease in the number of muscle fibers is the main cause of sarcopenia, although fiber atrophy is also involved.

A decline in strength of around 30% is observed in people with ages ranging from 50 to 70 years. These changes in the muscle structure are more common in women than in men, in the lower limbs than in the upper limbs and most of this decrease is caused by a selective atrophy in type IIB muscle fibers (American College of Sports Medicine, 1998).

However, it is believed that the aging process is responsible for the loss of α motoneurons; therefore, elderly individuals would show smaller amounts of motor units. This is explained by the degeneration of neural elements, re-organization of the other components, variation in the ratio of different types of motor units and alterations in the propriety of each motor unit.

Other physiological factors also contribute for the development of sarcopenia in advanced age, such as the decreased production of anabolic hormones, which jeopardizes the musculoskeletal capacity to incorporate aminoacids and to perform the protein synthesis. An increase in the release of catabolic agents also increases the muscle wear in seniors causing a decreased supply of glycolytic enzymes and smaller supply of ATP (Deschenes et al., 2004).

Studies have shown that the muscle mass starts to decrease in approximately 1% a year after the fourth decade of life. Most of the times, sarcopenia is marked by the stability of weight, due to the changes related to age in the body composition. However, several groups have reported the prevalence of sarcopenia, but these findings need to be further researched since they use different techniques to measure the lean mass and also use populations of different references. The prevalence of osteopenia and osteoporosis were estimated as 42% and 17%, respectively in women over 50 years old, where caucasian women showed the greatest number of cases of low bone density. Since the proportion of elderly older than 65 years in the population might increase, the incidence of sarcopenia and osteopenia might also increase. In women, menopause has been associated to a reduction in lean mass (LM) and bone mineral density (BMD). Several researches have demonstrated a positive relationship between LM and BMD and females with osteoporosis have been shown to have a significantly lower appendicular skeletal muscle mass compared to control groups. Based on the theory that the muscle mass is an indicator of BMD, one might speculate that sarcopenia is a risk factor for the development of oestopenia and that it is more prevalent in osteopenic individuals (Walsh et al., 2006).

Studies conducted by Walsh et al., 2006, revealed that 12.5% of postmenopausal women were osteopenic and that 25% of those postmenopausal osteopenic women and 50% of postmenopausal women with osteoporosis have sarcopenia. Therefore, they might present a higher risk of fractures compared to osteopenic women and osteoporotic women with a relatively normal skeletal muscle index.

Possible neural mechanisms that evidence this decrease in power associated to aging include the undefined changes in the CNS, a delay in the conduction velocity of motor nerve fibers and a delayed transmission in the neuromuscular junction or all three (Krueger et al., 2001). Similarly, a decrease in the number or the relative cross-sectional area of type II fibers, alterations in the sarcoplasmic reticulum and metabolism of calcium within the fibers, changes in the composition of isoforms of myosin in different fibers, functional and enzymatic properties of the myosin, an increase in the non contractile tissue, generating a greater resistance or combination of factors, might be responsible for the decreased power in the elderly (Hunter et al., 2004; Krueger et al., 2001).

The reduced capillary density and blood flow, impairment of glucose transport and lower mitochondrial density, decreased activity of oxidative enzymes and reduced rate of phosphocreatinine repletion contribute to the decrease in muscle endurance verified in people with advanced age (Krueger et al., 2001)

The loss of power might cause more damage to the elderly than the loss of maximum muscle strength since the development of explosive force is an important mechanism to prevent falls and to perform heavy duties such as velocity in rising from a chair and walking (Krueger et al., 2001; Hunter et al., 2004).

A fall can be defined as a sudden, unintentional change in position causing an individual to land at a lower level in relation to his initial position (Feder et al., 2000).

Almost all hip fractures occur as a result of a fall. These fractures are related not only to a decreased bone mass but also to factors such as a reduction in balance, strength and muscle

power in the lower extremities (American College of Sports Medicine, 1995; Nyberg et al., 1996).

The pathogenesis of fall is multifactorial (Nevitt et al., 1989; Tinetti et al., 1989). According to the Brazilian Society of Geriatric and Gerontology, 2008, the causes for falling might be divided in intrinsic and extrinsic and they are the following:

2.1 Intrinsic risk factors

- Previous history of falls – One or more falls in the previous year increase the risk of new falls in the subsequent year;
- Age – The prevalence of falls increases with age, however a review has shown that from 11 studies, only four found a positive association between aging and future falls;
- Females – In older women, the rate of women who fall is greater than in men and shows a greatest risk of fractures;
- Medications – Medications such as psychotropic drugs, cardiac medications like diuretics, antiarrhythmic, vasodilators and cardiac glycoside and polipharmacy (simultaneous use of four or more medications) are predisposing factors;
- Clinical condition – Diseases such as systemic arterial hypertension, diabetes mellitus and neurological or osteoarticular diseases affecting muscle strength, balance and gait are common risk factors. Orthostatic hypotension might be systematically researched due to its high prevalence. Severe diseases or unbalanced chronic conditions that affect the brain perfusion might also trigger a fall;
- Gait and balance disorders – They might be caused by aging itself, predisposing to falls when there is a decrease in force and endurance below the minimum threshold to perform independent daily life activities;
- Lack of physical exercise – The lack of physical exercise might cause an important musculoskeletal disorder;
- Psychological state – The fear of falling again after a fall is correlated to the worse performance of gait and new episodes of fall, which might restrict physical and social activities. Depression is also correlated to falls;
- Nutritional deficiency – It is related to the gait disorder, loss of muscle strength and osteoporosis;
- Cognitive impairment – Even a small deficit might increase the risk of fall;
- Visual impairment – Changes in acuity and visual field, as well as cataracts, glaucoma and macular degeneration are correlated to the increased risk of fall;
- Orthopedic disease – Diseases such as cervical spondilosis that might provoke dizziness, unbalance and feet problems, such as callus, deformities, ulcers and pain when walking also contribute to the genesis of fall;
- Functional state – the risk of falling is progressively increased according to the individual degree of dependence;

2.2 Extrinsic risk factors

The participation of environmental risk factors might reach, according to studies, up to 50% of the falls in elderly that live in the community. These factors include poor lighting, slippery surfaces, loose or folded rugs, high or narrow stairs, obstacles in the way (low

furniture, small objects, wires), lack of rails in halls and bathrooms, extremely low or high shelves, inadequate shoes and clothes, poorly maintained streets with holes or irregularities and inappropriate orthosis.

3. Exercise prescription

Intensity, duration, frequency and progression of the training are arguable, therefore future studies with better designs are required to evaluate these variables. Below are the exercise prescriptions for the elderly based on some consensus found in the literature:

3.1 Pre-participation

In general, the counter-indications are similar to the ones for a young adult. However, the need of a stress ECG is contradictory and it should be considered for patients with cardiac risk factors.

3.2 How to start

The exercises might have as a purpose to improve the functional limitations that seniors might have (pain, reduced movement range or muscle weakness). As soon as the limitations are improved, a program of general conditioning should be implemented to improve health and functional capacity of the elderly.

Training sessions should include three stages: warm-up, which involves low impact exercises to gain joint range of motion, training period (the effort itself), that involves muscle strengthening and/or aerobic exercises and the final stage that consists of stretching (cool down).

3.3 Stretching

Stretching should be performed during the warm up and in the last phase. A great joint range of motion (ROM) increases the muscle, reduces the risk of lesion and increases the cartilage nutrition. Painful joints should not be stretched excessively to a point that will result in more pain; all movements should be made in order to get the maximum pain-free ROM. The use of heat before stretching reduces pain and increases the range. At least three sessions of stretching might be performed a week. In the beginning, three to five repetitions and a gradual increase up to 10 repetitions is the ideal. The muscle should be stretched during 10 to 30 seconds.

3.4 Muscle strengthening

Muscle strengthening should be acquired with weights or elastic bands which will give endurance to the movement. The training protocols should include the following principles:

- muscle contraction exercises should be made in a moderate speed;
- exercises should be chosen according to joint stability and degree of pain and edema;
- muscles should not be exercised to fatigue;
- exercise endurance should be submaximal;
- inflamed articular joints should be strengthen with isometric exercises and at first it should include few repetitions;
- pain or edema in a joint after an hour of exercise indicates excessive activity.

- Isometric exercises are indicated for unstable or swollen joints. On the other hand, isometric contractions result in a low articular pressure and are well tolerated by older patients. It should start with contractions with an intensity of approximately 30% of maximal strength, slowly increasing to 80%. The contraction should not be kept for more than 6-10 seconds and the repetitions should be increased from 8 to 10, if tolerated by the patient. It should be performed twice a day during the inflammatory period and after the inflammation is over, it should be increased from 5 to 10 times a day.
- Isotonic exercises should include from 8 to 10 exercises involving the major muscle groups (four exercises for the upper limbs and from four to six for the lower limbs). At first, patients should use weights with 40% of the individual's maximal load, increasing up to 80%. Generally, a series of four to six repetitions should be made, avoiding the muscle fatigue. At first, the frequency should be at most twice a week but in case of individuals with advanced age or significant fragility the exercises should be made only once a week. Between the sessions, there might be at least one full day of rest.

4. Physical exercise to prevent falls

Prevention in individuals older than 60 years has an important role in avoiding adverse consequences resulting from falls (Weatherall, 2004).
The work to prevent fractures related to osteoporosis should focus the prevention or increase of material and structural properties of the bone, the prevention of falls and improvement of total mass of lean tissue (American College of Sports Medicine, 1995).
The American College of Sports Medicine recommends that:

1. physical activity of transporting weight is essential to the normal development and maintenance of a health skeleton. Activities that focus the increase of muscle strength might also be beneficial, particularly for bones that do not support weight;
2. a sedentary woman might progressively increase her bone mass by becoming active, but the primary benefit of increasing the activity is to prevent a future bone reduction that resulting from the lack of activity;
3. exercise should not be recommended as a replacement to medications treatment;
4. the optimal program for an older woman might include activities that improve the strength, flexibility and coordination which might indirectly, but effectively decrease the incidence of osteoporotic fractures by reducing the probability of falls. Therefore, the treatment of osteoporosis should aim the prevention of falls and fractures and preservation or improvement of bone mineral density.

4.1 Exercises for postural control

Postural control is a result of the combination of several types of sensorial information, such as visual, vestibular and somatosensorial information, and passive and active properties of the nervous system and skeletomuscle system that composes the human postural control system (Figure 2), (Shumway-Cook et al., 2000).
The postural control system use three functions that are required to maintain balance: support, stabilization and balance. The body should contract the adequate muscles to sustain the body against gravity; the articular segments should be stabilized and the body should be stabilized in the body's support base (Rothwell, 1994).
Currently, proprioception is defined as a set of afferent information provided by joints, muscles, tendons and other tissues that reaches the Central Nervous System (CNS) where it

is processed, having an influence on reflex responses and voluntary motor control. Proprioception contributes to postural control, joint stability and several conscious sensations (Lephart & Fu, 2000).

It is extremely important to understand that proprioception is only limited to the acquisition of the mechanical stimulus and its transduction in neural stimuli, not having any influence on the CNS processing and its motor response (Lephart & Fu, 2000).

Proprioception is part of a system denominated somatosensorial system. This includes all mechanical information provided by the mechanoreceptors. The feeling of pain is provided by the nociceptors and the thermal information provided by thermoreceptors (Guyton & Hall, 2006).

All propriocetive information are originated at the muscular and tendon receptors called muscular fusion and Golgi tendon organ and receptors located in ligaments, articular capsule, meniscus and cutaneous tissues (Guyton & Hall, 2006).

Four elements should be focused to reestablish the sensorimotor deficits: proprioception, stabilization, reactive neuromuscular control and functional motor patterns (Lephart & Henry, 1995).

The proprioceptive mechanism comprises both conscious and unconscious pathways. Therefore, the prescribed exercises need to include conscious exercises to stimulate the cognition as well as sudden and unexpected alterations of joint position that initiate reflex muscle contraction. These exercises should involve balance in an unstable surface while the individual perform functional activities. The purpose of the dynamic stabilization training is to improve the co-activation between the antagonist muscles (Hurd et al., 2006)

Exercises to stimulate proprioception and dynamic stabilization should be performed in closed-chain activities and with small movements, since the compression stimulates the articular receptors and the changes in the curve length-tension stimulate the muscle receptors. Limbs repositioning exercises should also be performed to stimulate the sense of joint position and neuromuscular control (Lephart & Henry, 1995).

The improvement of dynamic stiffness is another important aspect. It is suggested that muscle receptors increase its sensitivity through the increase of dynamic stiffness (Adler et al., 2008).

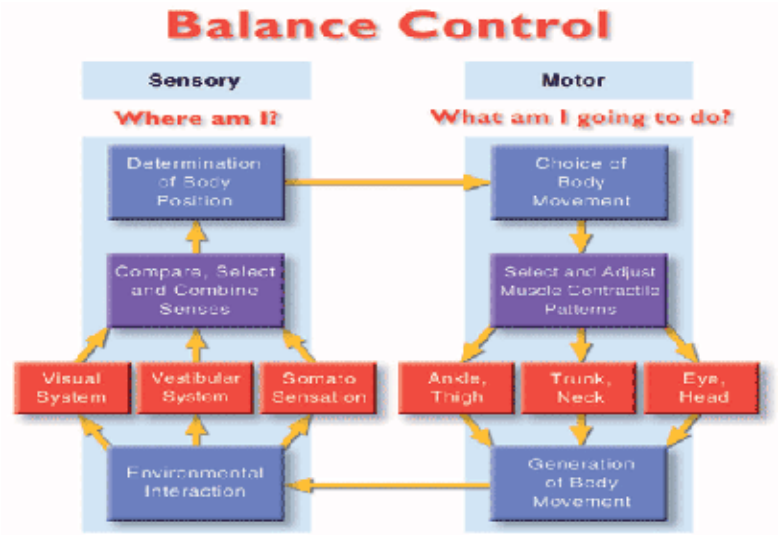

Fig. 2. Balance control: Sensory and motor system. Credit: http://resourcesonbalance.com

Exercises that involve eccentric training, like going down the stairs and landing after jumps, are the most efficient to increase anticipatory and reactive muscular stiffness (Bastian et al., 2006).

The reactive neuromuscular control is reached through exercises that create unexpected situations, such as perturbations in unstable surfaces in unipodal support and during gait. Apparently, this kind of training improves the preparatory and reactive muscle activation (Swanik et al., 2002).

The training protocol might include:

1. 5 – 10 minutes of warm-up, with stretching movements for upper and lower limbs, 03 repetitions for each movement being kept for 30 seconds, with 30-second intervals among the series. After stretching, movements of fast gait as previous warm-up were performed and in the end of the session, slow gait movements and stretching.
2. Proprioceptive exercises followed an evolution sequence based on the use of stable surfaces to unstable, walking straight forward progressing to changes in direction, from gait with no obstacles to gait with obstacles, alteration in the support base (from open to closed), exercises with eyes open to closed eyes, always respecting the functional capacity of each patient and progressively increasing the difficulty of each exercise. To aid the training, cones, balance boards, sticks, mats and trampolines were used. According to the patient's evolution, the exercises were combined creating the circuits (Figure 3).

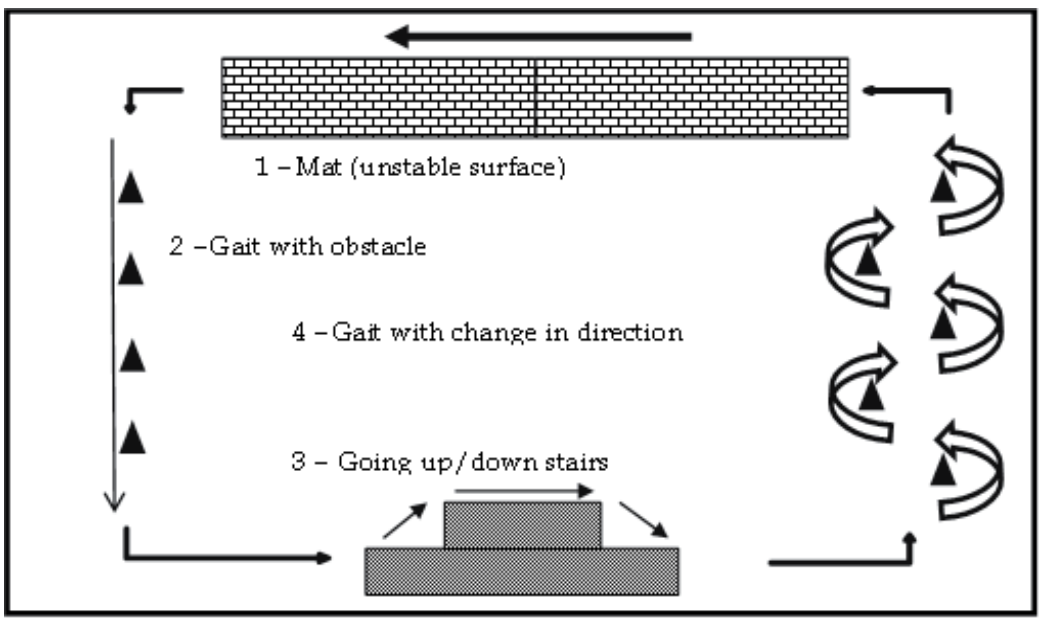

Fig. 3. Example of a circuit training

Examples of exercises: ten repetitions with one-minute intervals for antero-posterior and latero-lateral gait; gait with obstacles (20 cm high); gait over mattress; going up and down the stairs; change in direction according to the sound stimulus; balance exercises lasting 30 seconds and with one-minute interval for unipodal and bipodal support on the floor

with eyes open and/or closed; change in floor for a more unstable surface such as a trampoline and balance board; exercises with dissociation of waist and use of a stick (Table 1).

Options of Exercises	Evolution of Exercises	Time or # of repetitions
Balance exercises (balance board, mini-trampoline. Dyna disc)	Eyes open or closed / stable or unstable	10 rep / 30s
Stability exercises	Unipodal or bipodal support / open or close base	10 rep / 30s
Anteroposterior and latero-lateral gait	With or without obstacle and Variation in speed	10 rep (3 m)
Mat exercises	Go up/down: 1 to 3 mats	10 rep / 3 series
Exercises on the stairs	Variation in speed	10 rep / 3 series
Exercises with sticks	With or without arm movements	10 rep / 3 series

Table 1. Examples of exercises

Evidences have shown that specific exercises might reduce the risk factors for falls and number of falls in older people (Lord & Clark, 1996; Robertson et al., 2001-1, 2001-2; Hartard et al., 1996).

In 2006, Carvalho stated that the main goal of the osteoporosis treatment is to prevent fractures and as 90% of the fractures resulted from falls, the fundamental part of fracture treatment is to prevent them. This prevention represents a great area of interest in researches on older people's health (Weatherall, 2004).

Because of the strong interaction between osteoporosis and falls, the selection of participants in protocols for the prevention of fractures should be based on factors related to bones and falls (Pfeifer et al., 2004).

The German Society of Sport Medicine and the American College of Sport Medicine also recommend that the ideal program for women with osteoporosis should include activities that improve strength, flexibility and coordination that might indirectly and more effectively decrease the incidence of osteoporotic fractures by the reduction in the probability of falls (Lange et al., 2005).

Data combined from three studies conducted by Gillespie et al., 2006, with a total of 556 women aged 80 years or older, who underwent to the same progressive muscular strengthening program, balance training and gait training indicate that this intervention decreased the number of individuals that fell during a year, having also reduced the number of injurious falls. Although the studies had methodological limitations, there is a determined consistency as for the decrease of falls in multiple interventions exercises (Gillespie et al., 2009). As for the physical exercise, we only know that it improves balance without a direct association with the decrease in the number of falls (Howe Tracey et al., 2009) and that

although the decline in muscle strength is a risk factor for falls, the muscle strength training could not be associated to the reduced number of falls (Sherrington et al., 2008; Gillespie, et al., 2009).

Few studies take into consideration the importance of the proprioceptive training as a fundamental and unseparable part of a muscular strengthening program. Mechanoreceptors located in the joints, tendons, muscles and neighbor tissue provide information to the Nervous System about the position and articular movements and about the forces generated in the muscles (Hurley, 2003; Van der Esch et al., 2007).

The knee proprioception is essential for the modulation and accurate activation of the muscle contraction, once the functional skill and muscular balance are strongly affected by the proprioceptive inaccuracy and muscle weakness (Van der Esch et al., 2007). Studies including patients with knee ligament lesions show that the proprioceptive training promotes additional sensorial information that contributes to the improvement in postural control (Bonfin et al., 2008). This relationship becomes even more important when the muscle strengthening program aims to improve the functional balance and prevention of falls.

The significant results found in the present research might be explained by the concern in following the ACSM recommendations when prescribing exercises, respecting the basic concepts of prescription exercises.

Additionally, one should take into consideration that the skill to develop muscle strength decreases with aging (Hakkinen et al., 1998) explaining the importance of the gradual progression (Adams et al., 1999). With sedentary elderly people, a period of adaptation and low working load for two weeks should be applied for further implementation of a loading progression protocol (American College of Sports Medicine, 2002).

Teixeira et al., 2010, after eighteen weeks of training, observed an average increase of 87.5% in the maximal dynamic muscle strength in the quadriceps (1-RM) in volunteers in the intervention group, which is similar to the results found by Humphries et al., 2000, showing an increase from 20 to 200% in the dynamic muscle strength of the quadriceps depending on the figures in baseline and time of training. This increased knee extension strength is significantly important since the knee extension strength is an independent risk factor for falls and fractures caused by osteoporosis (Nguyen et al., 1993). The increase in strength results from neural alterations and muscle adaptations (Resende et al., 2008).

The combination of muscle strength and proprioceptive training was fundamental for a research that included postmenopausal women with osteoporosis conducted by Teixeira et al., 2010. The authors found an increase in mobility and functional capacity that might be related to a 36% decrease in time for performing the timed up & go test. We could observe that the shorter the time spent to perform the test, the better the balance (Resende et al., 2008). In this research, Teixeira et al., 2010, observed an improvement in balance evaluated by the Berg Balance Scale, where although there were small numerical changes, it was consistent, agreeing with the outcomes found by Madureira et al., 2006.

Bemben et al., 2000, compared the effects of high and low-intensity training in 25 postmenopausal women (41 to 60 years old) using a high repetition (40% 1-RM, 16 repetitions) and high load (80 % 1-RM, 8 repetitions) protocols for six months showing increases from 30 to 40%, respectively in the dynamic strength in quadriceps.

In a randomized controlled trial of 10 weeks of strength, balance and stretching training in 53 postmenopausal women with osteoporosis, Malmros et al., 1998, showed that strength and muscle mass and also the static balance improved significantly.

In another randomized clinical trial, physiotherapy-directed exercise in 30 patients with osteoporosis significantly improved static balance measured by functional reach and increased quadriceps dynamic strength (Mitchell et al., 1998).

These two studies indicate that the exercises programs improved the profile of fall risk but showed limitations because of the small number of samples and short time of the interventions.

Hartard et al., 1996, studied the effects of muscle strength training in 16 postmenopausal women with osteopenia, where fifteen belonged to the control group. Although they used a small group, a proper load protocol for 6 months, twice a week at 70% 1RM was applied demonstrating a considerable increase in muscle strength ranging from 44 to 76%, with results similar to the ones found in the present investigation.

Kemmler et ak., 2002, evaluated the dynamic force (1RM tests) in 137 postmenopausal women with osteopenia divided in two groups and observed a significant increase of 43% in the leg press in the intervention group training at 70% of 1-RM for fourteen months.

Carter et al., 2001, in a program that trains instructors to work with the community selected 93 postmenopausal women with osteoporosis who were randomized and underwent physical exercises of balance and muscle strength for twenty weeks. No improvement in the quality of life was found, which might be explained by the high quality of life at baseline. Researchers observed an improvement of 6.3% in the dynamic balance and an increase of 12.8% in the muscular strength.

On the other hand, Teixeira et al., 2010, showed a significant improvement in the quality of life evaluated by SF-36, where the values (regarding the physical aspects as well as mental aspects) were considerably superior than the controls and values at baseline. These results might be related to the systemic physiologic benefits provided by training, resulting in a better skill to perform daily life activities. We also related these results to the psychological effects of training, socialization with other patients and low initial levels of quality of life.

Madureira et al., 2006, conducted a randomized clinical trial that included 66 postmenopausal women with osteoporosis assigned to two groups. One of the groups underwent a 12-month of balance training once a week combined with oriented training at home showing significant results concerning balance, mobility and decrease in the number of falls.

Swanenburg et al., 2007, studied 24 women (65 years old or older) with osteoporosis or osteopeny who underwent three months of strength, balance and coordination training. After twelve months, they observed a reduction in the risk of fall (Berg Scale) and increase in the muscle strength of lower limbs. They also found a decrease in the number of falls in the intervention group (89%), showing a significant number although it was a pilot study.

As for the reduction of the risk of fall, although it shows an average of 40% (Barnett et al., 2003; Teixeira et al., 2010) it still is not well evidenced, which might be explained by the use of different populations and mainly the interventions used.

Several studies have shown to be effective in increasing the strength, improving the balance and functional capacity and decreasing the risk of falls (Table 2). Only the researches carried out by Madureira et al., 2006, Swanenburg et al., 2007 and Teixeira et al., 2010, directly associate these results and the number of falls demonstrating how effective these interventions were.

Muscle Strength Training				
Author	**Period**	**Method, intensity and volume**	**Sample**	**Results**
Hourigan, et al., 2008	20-weeks	In this study, subjects were randomised via computer-generated random numbers lists into either a control (receiving no intervention), or exercise group (two one-hour exercise sessions per week for 20 weeks with a trained physiotherapist).	Ninety-eight (98) community-dwelling osteopenic women aged 41-78 years	At the completion of the trial, the intervention group showed markedly significant better performances in balance (unilateral and bilateral stance sway measures, lateral reach, timed up and go and step test) ($p < 0.05$) with strong positive training effects reflecting improvements of between 10% to 71%. Similarly, there were gains in strength of the hip muscles (abductors, adductors, and external rotators), quadriceps and trunk extensors with training effects between 9% and 23%.
Teixeira, et al., 2010	18-weeks	The authors performed a study and randomized the sample into two groups: the intervention group comprised of 50 patients who underwent a 18-week of progressive load training for the quadriceps muscle (50% up to 80% of 1-RM-one maximum repetition) and proprioception training associated to a drug treatment of osteoporosis and the control group that included 50 patients who only underwent a drug treatment of osteoporosis. The muscular strength, balance, functional mobility, and quality of life were evaluated in the beginning and end of the research. The number of falls was evaluated 24 weeks post-treatment.	One hundred sedentary postmenopausal women with osteoporosis, ages ranging from 55 to 75,	The authors found out that the program promoted a significant difference among the groups for SF-36 in the eight sub-scales (p <or= 0.0018), Timed Up & Go Test ($p < 0.0001$), 1-RM test ($p < 0.0001$), Berg Balance Scale ($p < 0.0001$) and also a decrease in the number of falls in the intervention group compared to control (IRR = 0.263, 95% CI 0.10-0.68, $p = 0.0064$).
Burk, et al., 2010	8-weeks	The authors randomized the sample into two groups: intervention group, in which exercises for balance and improvement of muscular strength of the inferior members were performed for 8 wks (n = 17, age 72.8 +/- 3.6 yrs); control group, which was women not practicing exercises (n = 16, age 74.4 +/- 3.7 yrs). At baseline and after 8 wks of treatment, postural control was assessed using a force plate (Balance Master, Neurocom), and muscular strength during ankle dorsiflexion, knee extension, and flexion was assessed by dynamometry.	Sample consisted of 33 women with osteoporosis	When compared with the control group, individuals in the intervention group significantly improved the center of pressure velocity ($P = 0.02$) in the modified clinical test of sensory interaction for balance test, center of pressure velocity ($P < 0.01$), and directional control ($P < 0.01$) in limits of stability test, isometric force during ankle dorsiflexion ($P = 0.01$), knee extension ($P < 0.01$), and knee flexion ($P < 0.01$).

Table 2. Studies that used different methods of muscle strength training

5. Muscle strength training and the use of vibration platform

Although factors as genetic, hormonal homeostasis and nutrition may be affect the bone mineral density, the level of physical activity seems to have an important influence on this variable. The physiological mechanism that explains the osteogenic action of physical activity is not clearly understood. The moment the bone is compressed; negative charges in the place compressed are generated and positive charges in other areas (Figure 4).

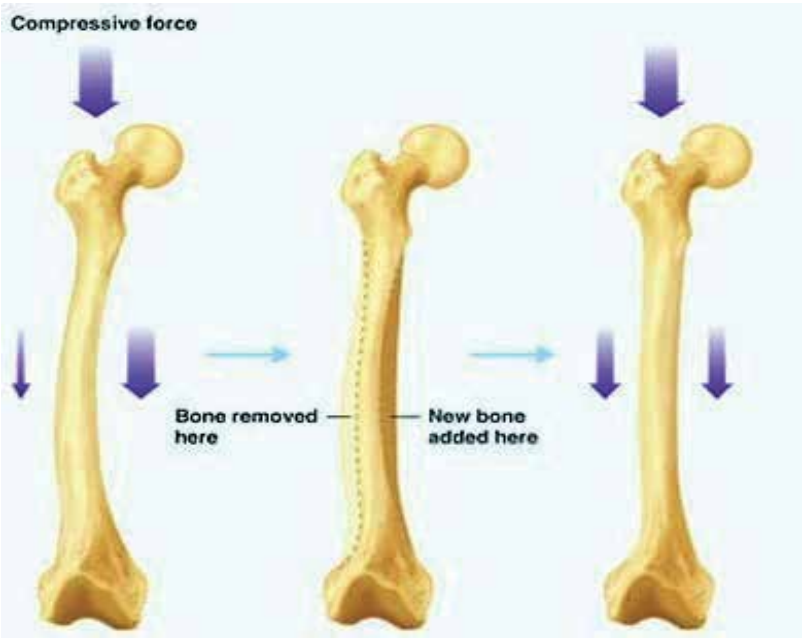

Fig. 4. a) The application of force to a slightly bent bone produces a greater compressive force on the inside curvatures. Compressive force producers weak electrical currents which stimulate osteoblast; b) Over time, bone is deposited in the inside curvature and removed from outside curvature; c) The final results is a bone matched to the compressive force to which it is exposed. Credit: Copyright, Person Education, Benjamin Cumings.

Minimal amounts of electric current stimulate the osteoblasts (bone-forming cells) in the negative extremity that is being compressed, increasing the bone formation in this area (Bankoff et al., 1998).

Another aspect that should be taken into consideration when ideally prescribing the strengthening training in order to stimulate the bone formation is the type of muscle contraction used. In studies comparing the eccentric and concentric strength training with the same relative load, the first showed to be more effective increasing the BMD (Hawkins et al., 1999; Hortobágvi et al., 1996; Aagaard et al., 2000).

The mechanism to increase the bone mineral density (BMD) through the strength training depends on the magnitude of bone deformation caused during this activity. In fact, higher-intensity training related to maximum load is generally associated to greater stimuli for the increase of BMD compared to low-intensity training (Kerr et al., 2001; Vincent & Braith, 2002). Besides that, the use of higher-intensity training implies in more immediate responses in the BMD.

Therefore, it can be concluded that in order to have a strength training providing beneficial effects over bone density, it is important to follow and respect some basic principles of physical training, such as proper overload, volume and intensity. On the other side, this training modality is the one that allows the greatest control of these variables.

5.1 Vibration platform

Vibration platform is a new type of exercise involving the application of a vibratory stimulus to the entire body as opposed to local stimulation of specific muscle groups (Merriman & Jackson, 2009) and has been increasingly tested for the ability to prevent bone fractures and osteoporosis in frail people (Gusi et al., 2006). It has become increasingly popular over the last several year as a form of physical training (Merriman & Jackson, 2009), since it is a non-pharmacological treatment alternative for osteoporosis (Cardinale & Wakeling, 2005). The platform can increase bone strength and bone mass (Sehmisch et al., 2009) since the vibration provides a low level of mechanical load stimulating, therefore the bone remodeling (Hannan et al., 2004). This can be explained by the combined effect on the neuromuscular and neuroendocrine systems (Cardinale & Wakeling, 2005). Vibrational physical exercise causes reflecting muscle contractions like tonic vibration reflex. This type of intervention leads to a high intensive stimulation of proprioceptors called muscle spindles which result in alteration in parameters of activity and development of human physiological functions (Piatin et al., 2009).

The vibrating devices currently marketed show two types of vibrating plates: a) the whole plate oscillates up and down; b) vertical displacements on the left and right side of a fulcrum, increasing the lateral accelerations (Gusi et al., 2006), (Figure 5). The units provide a vibration by using either a rotational or vertical stimulus, that is, the platform rotates about an anterior-posterior axis so that the positioning of feet further apart results in increased amplitude of movement and applies force asynchronously to the left and right foot, similar to standing near the middle of a 'teeter-totter. Vibration units that provide a vertical stimulus have a platform that translates vertically and symmetrically causing simultaneous movement of the lower extremities in the same direction. In addition to the duration of the vibration stimulus, there are several treatment parameters that are important to consider. These include frequency (Hz), amplitude (mm), duration and vibration magnitude (g), which is a gravitational acceleration imposed on the body. However, some studies have used frequencies ranging from 25-50 Hz, amplitudes from 2-10 mm, and total durations of 30 sec — 10 minutes. Currently, there is no consensus regarding the correct parameters needed to achieve a specific physiological response (Merriman & Jackson, 2009). However, some researchers have used frequencies ranging from 15-35 Hz to obtain a maximum transmissibility of the mechanical stimulus produced by the vibratory plate. Some recent studies have included in their protocols 15/10-Hz frequencies to allow a smooth adjustment in individuals considered frail, like the elderly (Gusi et al., 2006).

The effects of this vibration have been studied extensively in occupational medicine, mainly in industrial settings. It has been shown that when the body undergoes chronically to whole body vibrations spinal degeneration is likely to be one of the deleterious outcomes. Symptom of low back pain has been shown to be the leading major cause of industrial disability in the population under the age of 45 years (Cardinale & Pope, 2003).

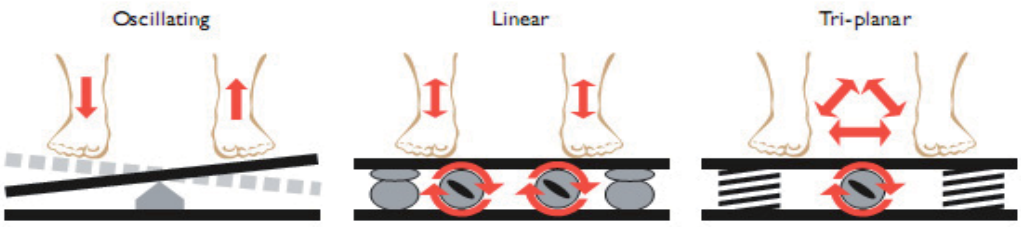

Fig. 5. Three different types of whole body vibration technology, including oscillating, linear and tri-planar platforms. Credit Larry Leggh. PhD and Jonathan Scherer MHK. J Active Aging. Nov/Dec 200

In a research conducted by Rubin et al., 2001, in adult rats, they found out that a combination of low magnitude and high frequency vibration significantly increased the anabolic activity of bone, bone density and specifically bone formation.
Studies (Torniven et al., 2003) in animals have shown that the vibrations might be an effective and safe way to improve mass competence and bone mechanic, providing a great potential to prevent osteoporosis.
High frequency (28Hz), very-low-magnitude vibration exercise has recently been reported to increase bone mass in experimental animals and in humans (Russo et al., 2003). Therefore, in order to obtain bone reinforcement, the frequency and amplitude of vibration should not exceed specified levels for the treatment. Furthermore, low-frequency vibration does not stimulate the bone sufficiently to cause significant remodeling (Aleyaasin & Harrigan, 2008).
Fractures are among the commonest and most expensive health problems in the elderly population, therefore the physical exercise is considered an effective and frequently recommended strategy. However, hard bone stress induced by the vigorous activity of weight bearing might increase the risk of lesions (Gusi et al., 2006; Gilsanz et al., 2006).
Although evidence is overwhelming that physical exercise positively affects muscle strength at all ages, compliance of older persons with traditional exercise programs is low, and only a small percentage of older persons exercise regularly (Russo et al., 2003). According to Liu et al., 2011, osteoporosis and its associated fractures are common complications of aging and that the purpose of most therapeutical strategies is to prevent and/or treat bone loss focused on nonpharmacological approaches. Therefore, aerobic exercise and/or whole-body vibration (WBV) might have beneficial effect on bone mass and provide an alternative approach to increase or maintain bone mineral density and reduce the risk of fracture (Table 3).
However, the mechanism through which the vibrations influence the bone tissue is still obscure. There is a lack of understanding the physiological mechanisms involved in the adaptive responses or the most appropriate vibration parameters to be used in order to maximize gains (Santin-Medeiros & Garatachea, 2010; Cardinale & Rittweger, 2006). The high-frequency postural displacements induced by the alternating movements of the platform produce reflex muscle contractions aimed at stabilizing posture. Thus, vibration can be viewed as a special form of muscle training that may particularly affect muscle power. It has been proposed that the force applied to bone during muscle contraction has a pivotal role in the homeostatic and adaptive regulation of bone strength (Russo et al., 2003). However, researchers (Torniven et al., 2003) carried out a study with the vibration platform and concluded there was no effect on the bones of young and healthy adults.

Whole-body vibration				
Author	Period of time	Method, intensity and volume	Sample	Results
Verschueren, et al., 2004	The WBV group and the RES group trained three times weekly for 24 weeks	The authors performed this randomized controlled trial to assess the musculoskeletal effects of high-frequency loading by means of whole body vibration (WBV) in postmenopausal women.	Seventy volunteers (age, 58-74 years) were randomly assigned to a whole body vibration training group (WBV, n = 25), a resistance training group (RES, n = 22) or a control group (CON, n = 23)	The authors found out that vibration training improved isometric and dynamic muscle strength (+15% and + 16%, respectively; $p < 0.01$) and also significantly increased BMD of the hip (+0.93%, $p < 0.05$). No changes in hip BMD were observed in women participating in resistance training or age-matched controls (-0.60% and -0.62%, respectively; not significant). Serum markers of bone turnover did not change in any of the groups.
Slatkovska, et al., 2010	Follow-up of ≥ 6 months	The authors performed a systematic review and meta-analysis where eligible RCTs included randomized or quasi-randomized trials, with follow-up of ≥ 6 months, examining WBV effects on BMD in ambulatory individuals without secondary causes of osteoporosis. The weighted mean differences between WBV and control groups in absolute pre-post change in spine and hip aBMD, and in spine and tibia trabecular volumetric BMD (vBMD) were calculated.	Eight RCTs in postmenopausal women (five RCTs), young adults (one RCT), and children and adolescents (two RCTs) were included. The regimens were heterogeneous, study durations were relatively short, and available data was mostly per-protocol.	In postmenopausal women, WBV was found to significantly increase hip aBMD (0.015 g cm(-2); 95% confidence interval (CI), 0.008-0.022; n = 131) versus controls, but not spine aBMD (n = 181) or tibia trabecular vBMD (n = 29). In young adults, WBV did not increase spine or hip bone mineral content, or tibia trabecular vBMD (n = 53). In children and adolescents, WBV significantly increased spine (6.2 mg cm(-3); 95% CI, 2.5-10.0; n = 65) and tibia (14.2 mg cm(-3); 95% CI, 5.2-23.2; n = 17) trabecular vBMD.

Table 3. Studies that used whole body vibration (WBV)

In a systematic review (Merriman & Jackson, 2009) conducted about the vibration platform to understand the effects on bone density, muscle performance, balance, and functional mobility in older adults concluded that most of the studies is methodologically weak and should be interpreted with caution. The study protocols use widely variable parameters which make the study interpretation difficult. The effects of this long term vibration (>1 year) still need to be studied. Some but not all of the studies in this review reported that individuals exposed to those vibrations showed similar improvements in muscle performance, balance, and functional mobility as compared to traditional exercise programs and that the vibration platform does not provide any additional benefit. Bone studies consistently showed that WBV improved bone density in the hip and tibia but not in the lumbar spine. Additional studies are needed to determine safe and effective parameters for WBV training in older adults.

However, the treatment has to follow specific safety guidelines to prevent vibration exercise-related injuries, such as limiting the exposure to vibration to a maximum of 10 minutes and maintaining a good posture of the participant.

Due to a great controversy in studies on its effects and parameters, more studies in humans with specific clinical recommendations and protocols are necessary for the vibration training (Gusi et al, 2006; Torniven et al., 2003).

6. Conclusion

Physical activity is an essential factor in bone health. The benefits of exercise have been demonstrated throughout the life cycle. Exercise can positively affect peak bone mass in children and adolescents; has been shown to help maintain or even modestly increase bone density in adulthood and; can assist in minimizing age related bone mass peak loss in older adults. Physical exercises that cause mechanical stress are the most recommended to increase or keep bone mass. However, the prevention of falls seems to be the most important factor in decreasing the risk of fractures in women with osteoporosis and in elderly people, since more than 90% of hip fractures results from falls.

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24

The Effect of Exercise on Bone Mineral Density, Bone Markers and Postural Stability in Subjects with Osteoporosis

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1. Introduction

1.1 Osteoporosis

Osteoporosis, as one of the major causes of disability, morbidity and mortality in older people, is currently considered as a global socioeconomic problem that is increasing in severity and frequency (Dontas & Yiannakopoulos, 2007).

1.1.1 Factors affecting osteoporosis incidence

The diagnosis of osteoporosis is based on the measurement of bone mineral density (BMD), which accounts for 70% of the bone strength, and is, therefore, a good indicator of an impending risk of fracture (Wilkins & Birge, 2005). According to World Health Organization, BMD values are divided into three groups: normal BMD (T-score up to -1.0 standard deviation (SD)), osteopenia (T-score between -1.0 and -2.4 SD), osteoporosis (T-score -2.5 SD and below). Wilkins and Birge (2005) presented these factors as contributing to reduced BMD: nonmodifiable – advanced age, female sex, white/asian race, family history of osteoporosis, family history of hip fracture, lactose intolerance, metabolic disorders affecting the skeleton, certain malignancies (myeloma, lymphoma), and modifiable – smoking, low calcium intake, low vitamin D intake/sunlight exposure, sedentary lifestyle, low body weight, stress/depression, surgical or drug induced hypogonadism, and glucocorticoid therapy.
The risk of fracture is considered as age-related. There are two main reasons: age-related decrease in bone mineral density of the proximal femur and the age-related increase in falls which is associated with worsening balance (Dontas & Yiannakopoulos, 2007).

1.1.2 Effect of physical activity on BMD

Intervention strategies for osteoporosis are based on a combination of pharmacological agents, nutrition and a suitable physical activity (Melendez-Ortega, 2007).
When determining the effect of movement (overcoming gravitational force) on the quality of bone tissue, three basic mechanisms are applied: activation of osteoblasts, storing Ca^{+2} ions

on the bone surface, and increase in the bone substance required for ossification (Němcová & Korsa, 2008).
The results of studies focused on the effect of physical activity on changes in the quality of bone tissue vary. Englund et al. (2005) present the direct effect of weight-bearing training programme on improvement in BMD. Kemmler et al. (2004), Kerry (2003), Uusi-Rasi et al. (2003) and others didn't find the effect of exercise on BMD in postmenopausal women. The results, which were presented in the study of Bloomfield (2005), indicate that exercise may minimize or even stop the bones losing weight in postmenopausal women. However, it does not substitute pharmacological agents nor does it ensure increase in BMD.

1.2 Balance

The human body in standing can be described as a naturally unstable system. The complexity and instability of this system is given by a large number of mobile segments (Véle, 1996) and also by the fact that in standing, 2/3 of body mass is at 2/3 of the individual's height above ground (Winter, 1995).

1.2.1 Factors influencing balance

Besides low bone mineral density, also poor stability contributes to increased risk of fractures associated with a fall (Winters & Snow, 2000).
The basic factors affecting balance include the quality of sensory inputs (vestibular, eyes, tactile, proprioreception …), neural control (central nervous system) and the effectors (muscles, bones …).
Poulain and Giraudet (2008) proved the existence of greater visual sensitivity in posture control from 44 years of age. They also showed that the measurement of the role of vision in posture control among subjects aged 44–60 years strongly depends on the task performed.
The effect of ageing and vision on limb load asymmetry during a quiet stance was observed by Blaszczyk et al. (2000). Their observations may indicate that increased limb load asymmetry in the elderly is a consequence of many kinds of compensatory changes in postural stability control.
Some studies have shown that postural stability is also affected by anthropometric parameters. Chiari et al. (2002) suggested that some anthropometric measurements and standardization or tracing foot position could be considered. The study by Hue et al. (2007) shows that increase in body weight correlates with higher balance instability.
The effect of local fatigue was observed by Caron (2004). The main result of this study was that local fatigue of the lower limbs produced similar effects on postural control and postural stability in the standing with a more pronounced increase in neuromuscular activity with the eyes open as compared to eyes closed.
Hlavačková et al. (2009) suggested that elderly people with some limb deficiencies (transfemoral amputees) were able to integrate augmented visual biofeedback through the use of mirror-reflected body image to improve their stance control during quiet standing.

1.2.2 Balance evaluation

Clinical assessment of balance

The main purpose of the clinical balance assessment is to identify whether or not a balance problem exists and whether treatment is needed (Horak, 1997).

An often used instrument for balance evaluation is "The Berg Balance Test". This test consists of 14 functional subtests with a maximum of 4 points (normal performance) and a minimum of 0 points (no performance) (Berg et al., 1989).
Horak et al. (2009) created "The balance evaluation systems test (BESTest)" to differentiate between balance deficits. This test encompasses six areas:

- biomechanical constraints,
- stability limits/verticality,
- anticipatory postural adjustments,
- postural responses,
- sensory orientation,
- and stability in gait.

Posturography

Instrumental measurement of balance can be performed by posturography, which, by means of force platforms, analyses the centre of pressure (COP) movement on various types of stands. The basic measured parameters are the area of confidence ellipse, the length of COP trajectory, sway of COP and its velocity in the antero-posterior and medio-lateral direction.
In recent years, the focus of attention has been especially on the character of a task being handled and on data analysis. When looking to predict falls, stability evaluation in quasistatic and dynamic situations is better. The basic tasks here are:

- reaction to tripping of a force platform,
- solution when using a dual task,
- testing the stability limits,
- targeted modification of sensory inputs,
- and testing a stand in varying conditions.

Measurement accuracy and reliability

A review of the test–retest reliability of the centre of pressure measurements in the bipedal static condition was presented by Ruhe et al. (2010). They mentioned that the reliability of the traditional COP parameters can be acceptable; however, it depends primarily on factors such as the number of trial recordings and the duration rather than the selection of particular COP parameters. They also recommend that care should be taken to assess the subject's physical status and anthropometric properties prior to the measurements.
Le Clair and Riach (1996) demonstrated that the test duration affects the measurement of postural sway, with 10 s being the least reliable. They also reported that COP, force, and velocity measurements are reliable in retest situations and that only one trial is necessary to obtain reliable measurements.
On the contrary, the results of the theory analysis (Doyle et al., 2007) suggest that COP measurements reached acceptable levels of reliability with at least five 60 s trials.

1.3 Fall risks

One of the biggest problems in people with osteoporosis is an increase in the risk of fall. Patients with osteoporosis do not have a good level of stability and have less muscular strength. This makes the risk of fall a lot higher than in individuals without this illness (Park et al., 2008). Regular balance, strength training and diet supplementation with vitamin D and calcium are important in falls prevention (Kannus et al., 2005).

Reduction of the risk of fall requires identification of the individual with risk of fall and identification of the modifiable risk factors (Wilkins & Birge, 2005).

Causes of falls

Four major categories of the causes of falls were defined by Lach et al. (1991):

- falls related to extrinsic factors,
- falls related to intrinsic factors,
- falls from a non-bipedal stance,
- and unclassified falls.

Extrinsic factors pertain to environmental hazards such as loose carpeting, stairs, and poor footwear or lighting. Intrinsic factors are conditions that relate directly to a specific person such as dizziness, use of medication, osteoporosis, and arthritis (Hale et al., 1992).

A comprehensive prospective study concerning the risks associated with falls in older men was performed by Chan et al. (2007). They found that leg extension power, grip strength, and activity level are significant determinants in assessing the risk of fall. The authors also reported increased activity being associated with higher risk of fall; household activities were associated with the risk whereas leisure activities were not.

Measuring falls

Hauer et al. (2006) presented a systematic review of the definitions and methods of measuring falls in randomised controlled fall prevention trials. Studies focused on assessing the risk of fall have brought forward many issues, one of them being an inconclusive definition of the term "fall" itself. Additionally, the method used in the studies to report falls remains problematic and highly inconsistent. For future research, they recommend a comprehensive and non-exclusive definition of a fall.

Evaluation of the risks of fall

In order to determine the risk of fall, it is important to firstly render an evaluation of the act. Brauer et al. (2000) presented a prospective study of laboratory and clinical measurements of postural stability to predict fallers in community dwellers. They found out that not all older adults with reduced or compromised balance ability reported a fall over a 6-month period. Therefore, they emphasized the importance of the multifactor nature of falls.

Lord et al. (2003) described the use of a physiological profile approach to falls risk assessment and prevention. This evaluation involves a series of simple tests of vision, peripheral sensation, muscle force, reaction time, and postural sway.

The effect of physical activity on balance and risk of falls

Hourigan et al. (2008) found an increase in strength of the hip joint muscles and trunk extensors by 9-23% as well as significant improvement in balance after 20 weeks of exercise. Wendlová (2008) states an increase in muscular strength, improved possibility of reacting to loss of balance and reduced risk of fall as a result of exercising. Vaillant et al. (2006) supplemented exercising for balance development with cognitive tasks. While exercising resulted in improved balance, the use of cognitive tasks brought no further changes.

Karinkanta et al. (2007) analysed four groups with different intervention (resistance training, balance-jumping training, combination of both trainings, and no training) criteria and concluded that training prevented functional decline in home-dwelling elderly women.

For osteoporosis patients, weight-bearing activities, balance exercise and strengthening exercises to reduce fall and fracture risk are recommended (De Kam et al., 2009).

The effect of three types of exercises (resistance training, agility training, and general stretching) on risks of fall and the physical activity level of women with low BMD was observed by Liu-Ambrose et al. (2005). They found significant decrease in the fall risk after a 6-month regimen. Even twelve months after intervention the risks of fall remained significantly lower than before exercising. It is very interesting to note that after all three types of exercise programmes, the benefits remained sustainable for at least 12 months. Thus, these 6-month exercise interventions appeared to act as a catalyst for increasing physical activity with resultant reduction in the fall risk profile.
Exercising leads, above all, to improvement in the medio-lateral direction and individuals are then able to control movement at the hip joints within a greater range and with better results (Nagy et al., 2007). Twiss et al. (2009) found improvements in balance after carrying out yearly exercises focused on the development of muscular strength and weight training. The number of falls in those exercising dropped, though insignificantly. Improvement in balance and quality of life after five weeks of exercising was recorded by Alp et al. (2007).
Multifactor preventive and individually-focused balance programmes may reduce the risk of falls in individuals by 25 to 30% (Dargent-Molina, 2004). Whereas Carter et al. (2001) recorded insignificant differences between the observed group and control group in static and dynamic balance after 10 weeks of exercise intervention.
Swanenburg et al. (2007) observed the effect of a three-month exercise programme that included training for muscular strength, coordination, balance, and endurance when accompanied with nutritional (protein) supplements. They mentioned that the combination of calcium/vitamin D and exercise/protein intervention programme significantly reduced the risk of fall and in addition, these effects lasted for up to 9 months after the end of the intervention programme.
Madureira et al. (2007) compared groups with and without the balance training programme. The percentage of patients in the intervention group whose static balance improved in two sensory conditions (eyes closed, unstable surface; and eyes open, visual conflict, unstable surface) was statistically significant when compared to the control group. Also, significant difference in the functional mobility as well as reduction in the number of falls/patient were observed in the intervention group.

2. Aim

The aim of this study was to assess the effect of exercise on bone mineral density and bone markers in postmenopausal women with osteoporosis and to determine the effect of a specific exercise programme for postural stability.

3. Material and methods

3.1 Characteristics of the group

The tested group included 163 women, patients of the Osteology centre in Zlín, who were randomly divided into groups to perform an exercising programme (n=90) and control (non-exercising) group (n=73). Through a combination of two factors (exercise, specific pharmacological therapy), 6 sub-groups were formed:

1. NEX/NS (n=23, age 57.8±6.05 years): non-exercising group with nonspecific pharmacological therapy (i.e. daily controlled intake of Ca 1000–1500 mg, daily controlled intake of vitamin D3 0.25 μg).

2. EX/NS (n=32, age 59.0±7.11 years): exercising group with nonspecific pharmacological therapy (i.e. daily controlled intake of Ca 1000–1500 mg, daily controlled intake of vitamin D3 0.25 μg).
3. NEX/BP (n=27, age 62.9±7.06 years): non-exercising group with specific pharmacological therapy, suppressing bone resorption (bisphosphonates – Fosamax, controlled intake of Ca, administration of vitamin D3).
4. EX/BP (n=35, age 59.7±7.56 years): exercising group with specific pharmacological therapy, suppressing bone resorption (bisphosphonates – Fosamax, controlled intake of Ca, administration of vitamin D3).
5. NEX/SERM (n=23, age 61.1±6.90 years): non-exercising group with specific pharmacological therapy (selective estrogen receptor modulators – Evista, controlled intake of Ca, administration of vitamin D3).
6. EX/SERM (n=23, age 59.0±6.53 years): exercising group with specific pharmacological therapy (selective estrogen receptor modulators – Evista, controlled intake of Ca, administration of vitamin D3).

3.2 Exercise

Exercise intervention was divided into three parts:

1. Three-week institutionalised exercise intervention in a rehabilitation centre focused on changing posture, adjustment of joint mobility, adjustment of muscle imbalance, coordination and postural correction, relaxing of shortened muscle groups and soft tissues, activation of muscles in the area of axis system, deep stabilization system and extremities, breathing exercises and coordination training, training for activity of daily living and training for walking.
2. Three-month controlled group exercise programme focused on motivating and educating patients towards active approach; once a week of low intensity and of 50 min duration.
3. Daily exercise programme at home lasting 30 minutes, supplemented with walking of minimum 60 min duration.

In the further phase of the research, we evaluated the effect of long-term exercise on postural stability. The observed group consisted of 43 women, which were divided into a group with long-term exercise programme (n=29, age 64.6±4.52 years) and the control group (n=14, age 65.5±4.98 years). Sample size was influenced by agreement of subjects with follow-up to research and limitations of workplace for application of the special exercise. The group went through controlled exercise intervention of low intensity for 50 minutes per week for a period of one year. The exercise unit was focused on maintaining and improving the quality of sensorimotor functions and postural strategies and was also completed by an hour daily home exercises. The progression of exercise consisted in repetition of a selected exercise pattern and in the change of position, i.e. transition from a lower to a higher position. The aim of the exercise in such easier and more demanding positions was to adjust and improve the quality of the respiratory mechanics, postural pattern and motor functions. Exercising in the vertical position was stipulated for adaptation to various types of stands, exercising balance strategies, and enabled an increased self-confidence for motion in space.

3.3 Measurement process, technical equipment, measured parameters

The bone mineral density was measured by the LUNAR-DPX device (GE Healthcare, Madison, WI, USA) and bone markers were determined by ETI-MAX 3000 (Diasorin S.p.A.,

Saluggia, Italy). The biochemical parameters were evaluated using VITROS 250 (Ortho-Clinical Diagnostics, Rochester, NY, USA) analyser.
For all patient groups, the baseline values of bone density (BMD L1-L4, BMD femoral neck) and bone markers (Ca, P, creatinine, ALP, ALP isoenzyme, osteocalcin, crosslaps) were measured at the beginning of research. For ascertaining the bone marker values, measurement was repeated after 3 weeks and 3 months. The last measurement in the range of baseline measurement was performed after 1 year of starting the research.
In the second phase of the research, all measured women repeatedly completed the six basic types of stands (eyes open, eyes closed, head extension, standing on foam, tandem stand twice), whereas each such stand was of 30 s duration. The feet position during such stand (with the exception of tandem stand) was set at pelvic width (the distance between the anterior superior iliac spines).
Two Kistler piezoelectric platforms, type 9286AA (Kistler Instrumente AG, Winterthur, Switzerland) were used for evaluating postural stability. The changes in the load on the lower limbs during standing as well as changes to the centre of pressure (COP) displacement (postural sway of COP and its velocity in antero-posterior and in medio-lateral directions) were determined by the software Bioware, version 3.2.6.104.

3.4 Statistical data processing

The measured data was processed by the Statistica 8.0 (StatSoft, Inc., Tulsa, OK, USA) programme. In order to compare the impact of exercise on BMD and bone markers, one-way ANOVA with Fisher's post-hoc test were used. The comparison of differences between the exercising and control groups upon evaluating postural stability was performed by means of t-test for independent groups. Any p-value less than 0.05 was deemed significant.

4. Results

4.1 Bone mineral density, bone markers

The basic statistical characteristics of the measured parameters for non-exercising groups as well as groups undergoing the intervention programme are stated in Tables 1 and 2.

BMD L1-L4 (Fig. 1)

During the monitored period, statistically significant increase in BMD L1-L4 value occurred in all measured groups with the exception of EX/SERM. A higher increase in BMD L1-L4 occurred in persons who did not undergo the targeted exercise intervention. The highest increase was recorded in NEX/BP and NEX/SERM. The difference in values between the groups with same medication is not statistically significant at baseline and after 1 year with $p<0.05$.

BMD femoral neck (Fig. 2)

Increase in the value of BMD femoral neck occurred in all measured groups with the exception of EX/NS. The extent of changes is comparable in the exercising as well as in the non-exercising patients. This increase is statistically significant in NEX/BP, EX/BP and NEX/SERM. Between groups with the same medication, there is a statistically significant difference at baseline and after 1 year measurements for NEX/BP (7.1%) and EX/BP (7.6%).

		NEX/NS (n=23)	NEX/BP (n=27)	NEX/SERM (n=23)
Age at baseline		57.8±6.05	59.7±7.56	59.0±6.53
Height (cm)		162.1±6.09	158.7±5.74	158.2±5.05
Weight (kg)	baseline	72.2±8.67	64.9±9.00	65.2±10.05
	1 year	73.6±8.68	66.1±8.65	65.2±9.37
BMD L1-L4 (g/cm^2)	baseline	0.99±0.089	0.92±0.110	0.93±0.089
	1 year	1.03±0.107	0.97±0.110	0.98±0.102
BMD femoral neck (g/cm^2)	baseline	0.93±0.115	0.91±0.115	0.87±0.082
	1 year	0.94±0.109	0.93±0.111	0.88±0.080
Calcium (mmol/l)	baseline	2.36±0.112	2.42±0.167	2.39±0.060
	1 year	2.38±0.113	2.41±0.133	2.48±0.154
Phosphorus (mmol/l)	baseline	1.11±0.164	1.12±0.232	1.11±0.205
	1 year	1.06±0.162	1.08±0.166	1.07±0.151
Creatinine (µmol/l)	baseline	81.2±10.21	83.2±10.06	78.4±11.16
	1 year	79.0±12.78	79.0±9.09	75.3±16.08
ALP (µkat/l)	baseline	1.09±0.409	1.28±0.481	1.17±0.383
	1 year	1.03±0.317	1.03±0.358	1.01±0.263
ALP isoenzyme (µkat/l)	baseline	0.54±0.008	0.53±0.011	0.54±0.006
	1 year	0.54±0.007	0.53±0.042	0.53±0.029
Osteocalcin (ng/ml)	baseline	25.0±19.20	30.0±26.51	21.3±12.72
	1 year	19.0±10.88	20.1±30.77	13.8±6.18
Crosslaps (ng/ml)	baseline	0.46±0.246	0.63±0.449	0.45±0.209
	1 year	0.37±0.190	0.42±0.652	0.31±0.167

Table 1. Characteristics of measured parameters for the non-exercising groups (Mean ± SD)

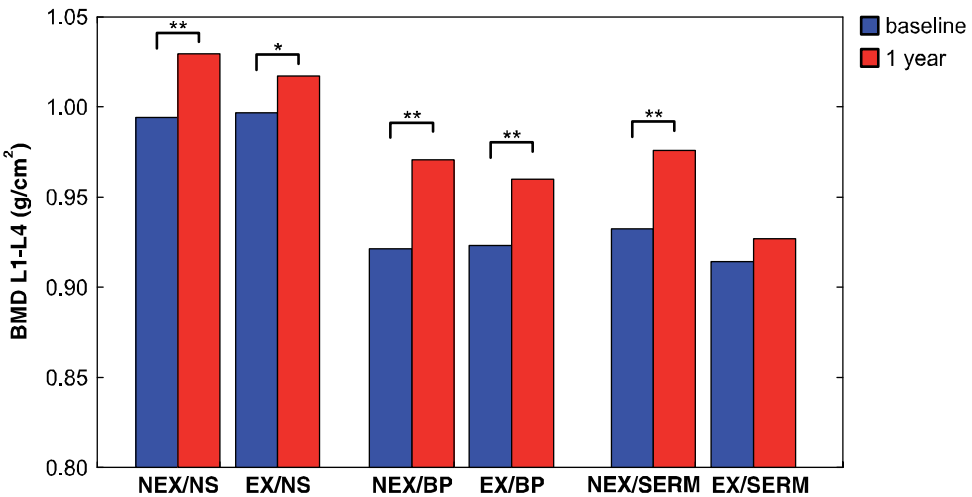

Fig. 1. Changes between the baseline and after 1 year measurements – BMD L1-L4 (* $p<0.05$, ** $p<0.01$)

		EX/NS (n=32)	EX/BP (n=35)	EX/SERM (n=23)
Age at baseline		59.0±7.11	62.9±7.06	61.1±6.90
Height (cm)		161.2±6.48	159.4±6.02	158.3±7.17
Weight (kg)	baseline	73.0±13.49	65.4±8.55	68.2±10.84
	1 year	72.6±13.36	66.0±8.50	68.4±11.01
BMD L1-L4 (g/cm^2)	baseline	1.00±0.079	0.92±0.114	0.91±0.147
	1 year	1.02±0.086	0.96±0.110	0.93±0.151
BMD femoral neck (g/cm^2)	baseline	0.94±0.093	0.85±0.123	0.85±0.148
	1 year	0.94±0.091	0.86±0.129	0.86±0.140
Calcium (mmol/l)	baseline	2.40±0.109	2.37±0.101	2.42±0.180
	1 year	2.38±0.093	2.41±0.158	2.34±0.112
Phosphorus (mmol/l)	baseline	1.02±0.162	1.02±0.129	1.05±0.130
	1 year	1.06±0.167	1.05±0.115	1.06±0.171
Creatinine (µmol/l)	baseline	80.0±12.46	81.7±9.13	79.4±9.60
	1 year	81.4±16.61	81.4±12.08	79.2±11.90
ALP (µkat/l)	baseline	1.15±0.437	1.09±0.320	1.10±0.309
	1 year	1.13±0.490	1.01±0.265	1.19±0.461
ALP isoenzyme (µkat/l)	baseline	0.54±0.005	0.53±0.007	0.54±0.006
	1 year	0.52±0.043	0.53±0.008	0.53±0.010
Osteocalcin (ng/ml)	baseline	14.7±10.55	17.6±17.72	18.0±10.44
	1 year	16.9±15.00	11.5±5.41	18.7±8.79
Crosslaps (ng/ml)	baseline	0.33±0.168	0.33±0.199	0.39±0.223
	1 year	0.38±0.198	0.27±0.170	0.45±0.187

Table 2. Characteristics of measured parameters for the groups performing the targeted exercise intervention (Mean ± SD)

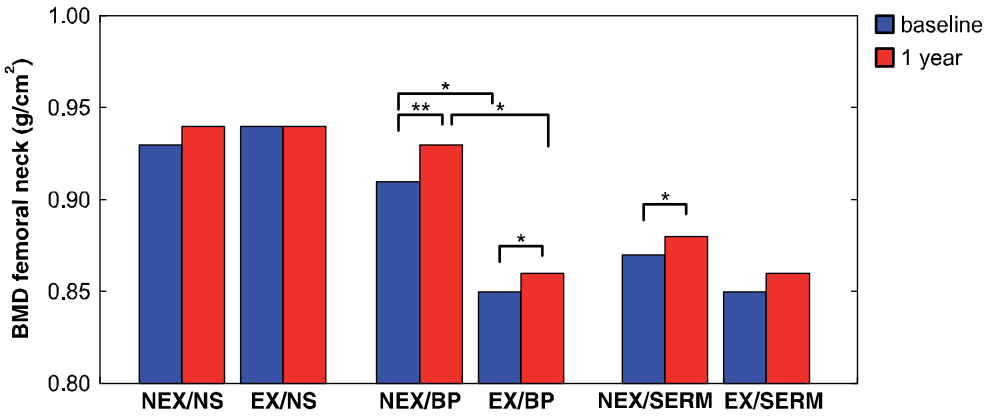

Fig. 2. Changes between the baseline and after 1 year measurements – BMD femoral neck (* $p<0.05$, ** $p<0.01$)

Calcium

Differences between baseline and after 1 year measurements are statistically insignificant, when compared for the exercising and non-exercising patients, with the exception of NEX/SERM. In this group, there was an increase in Ca during the entire period of monitoring; differences are also significant in the measurements after 3 weeks or 3 months in comparison with the values measured after 1 year.

Phosphorus

When comparing groups with the same medication, the value measured at baseline was significantly lower in groups of patients that were starting exercise intervention. Such difference was not found in any other measurements.

Creatinine

In NEX/BP and NEX/SERM, statistically significant decrease ($p<0.05$) in the value of this parameter occurred after 1 year. Throughout the entire period, no significant differences were found in groups undergoing targeted intervention.

ALP (Fig. 3)

In NEX/BP and EX/BP decrease in ALP value occurred during the monitored period. Differences between baseline and after 1 year measurements are significant (NEX/BP, $p<0.01$). A similar result is also valid for NEX/SERM where the parameter had a declining tendency throughout the entire monitored period.

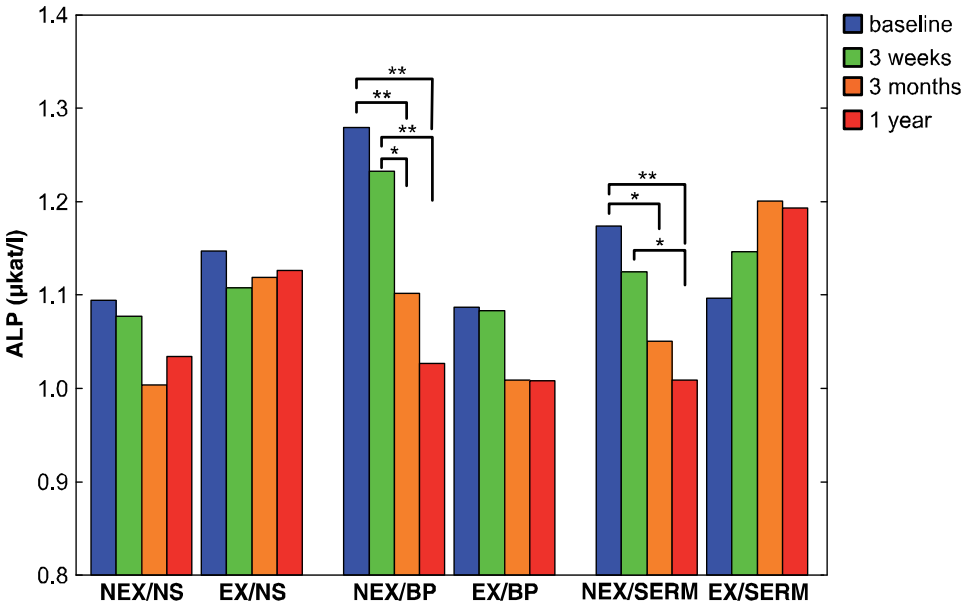

Fig. 3. Changes of the ALP in the course of the monitored period (* $p<0.05$, ** $p<0.01$)

ALP isoenzyme

With the exception of EX/NS, differences in the values between individual measurements in all other groups were not statistically significant at level $p<0.05$.

Osteocalcin (Fig. 4)

In groups with the same medication, the value at baseline is higher in patients, who did not undergo exercise intervention. No significant difference was recorded at measurement after 1 year. Throughout the monitored period, a significant reduction in this parameter occurred in NEX/NS, NEX/BP and NEX/SERM. A similar result was also valid for EX/BP.

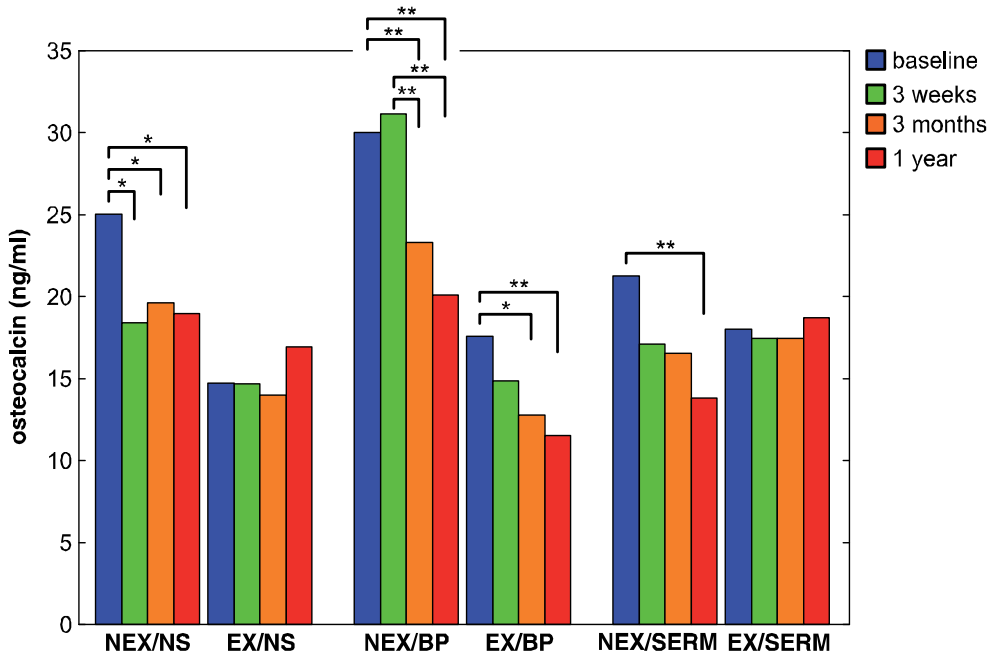

Fig. 4. Changes of osteocalcin in the course of the monitored period (* $p<0.05$, ** $p<0.01$)

Crosslaps

At baseline, the parameter was significantly higher in patients who did not undergo exercise intervention in comparison to the group of exercising patients. This difference was not recorded at measurement after 1 year. The decrease in value for the monitored period is significant for NEX/BP ($p<0.05$) and NEX/SERM ($p<0.01$).

4.2 Postural stability evaluation

No differences in weight-bearing distribution between the left and right lower extremity were found in any measured group or individual type of stand. Differences vary between 0.09 and 0.56% in the group of exercising and between 0.13 and 1.68% in the group of non-exercising patients.

Amongst both groups, we found no significant differences in COP sway in any directions measured for individual types of stand. In the group of non-exercising patients, faster change in COP position in the medio-lateral direction ($p<0.05$) occurs when standing with eyes closed, and such changes are reflected in higher resulting velocity of COP movement ($p<0.05$). A similar result applies to the velocity of COP in the antero-posterior direction, and for the resulting velocity of COP when standing on foam (Fig. 5).

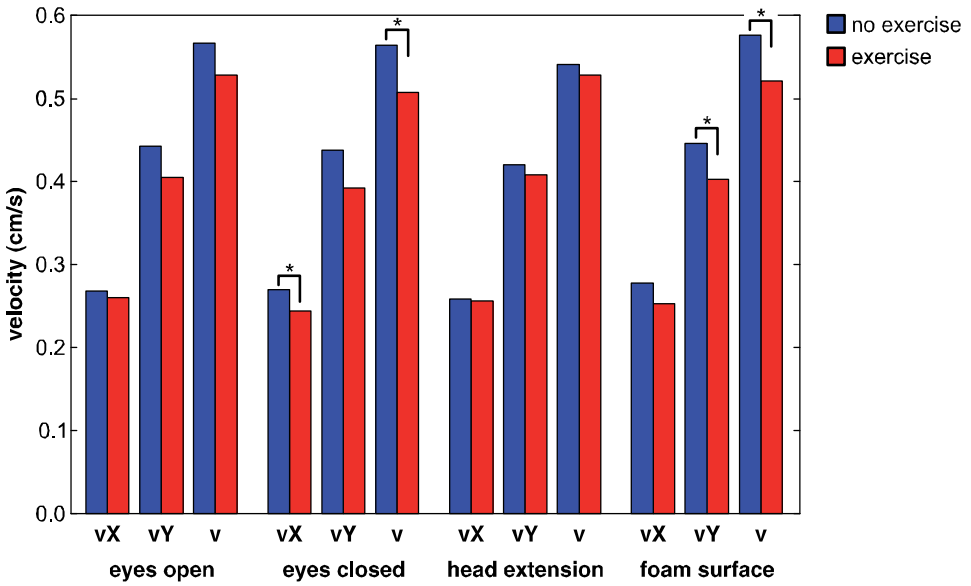

Fig. 5. The COP velocity in different types of stands for exercising and non-exercising patients (vX – COP velocity in medio-lateral direction, vY – COP velocity in antero-posterior direction, v – resultant COP velocity, * $p<0.05$, ** $p<0.01$)

With the exception of standard deviation for COP movement in the antero-posterior direction at stand with eyes open and stand on foam in the group of exercising patients, we found no significant differences ($p<0.05$). The exclusion of visual control or head extension thereby did not reflect in the change(s) of the measured parameters.

5. Discussion

5.1 Exercise, medication and bone mineral density

In order to maintain desirable optimal amount of BMD, it is required to keep an optimal level of tension relating to functional load given by physical activity (Melendez-Ortega, 2007). Mechanic load, induced in the course of exercise, is essential for adaptation of bone architecture in the place of the load (Vainionpää et al., 2005). That is the reason why movement is a significant element in prevention and treatment of osteoporosis.

5.1.1 Effect of exercise on postmenopausal bone

From the results of our study follows that BMD was increased in all participants who were involved in the exercise intervention. The changes are, however, not significantly higher in comparison with the non-exercising groups. For bone density of the colli femoris (BMD femoral neck), the increase was smaller than in the area of lumbar spine (BMD L1-L4) for all types of medications.

Opinions of authors concerning the effect of exercise on the quality of bone tissue vary. The effect of exercise on regional BMD in postmenopausal women was evaluated by Kelley (1998). Across all designs and categories, treatment effect changes in bone density ranged from -17.10 to 17.30%. Meta-analytic review of included studies suggests that exercise may slow the rate of bone loss in this group of patients.

The results of Angin and Erden (2009) demonstrate positive influence of the exercise program in increasing BMD and quality of life. The efficacy of a 5-year exercise program on the BMD and balance was investigated by Walker et al. (2000). For the post-menopausal women with osteoporosis who participated in the program it was possible to stabilize the BMD of the lumbar site, and to reduce fractures. Lange et al. (2007) presented that physical activity has a decelerating effect on the bone loss rate in postmenopausal women, independent of hormone replacement therapy. A significant increase in BMD and decrease in bone markers was found by Ďurišová and Zvarka (2004) in both exercise and control group. Authors state that regular exercise should become an important component of the comprehensive management of osteoporosis. Bergström et al. (2008) indicated a positive effect of physical training on hip BMD. No significant effect of exercise was found in the lumbar spine.

The effect of exercise on BMD can be influenced by its type, intensity and frequency. Kerr et al. (1996) examined the effect of a 1-year progressive resistance training program (strength and endurance group) on bone mass. They state that postmenopausal bone mass can be significantly increased by strength exercise with high-load low repetitions. For an endurance regimen this change was not established. On the contrary, Bemben and Bemben (2011) found positive BMD responses for the hip and spine (not for the total body) for all types of resistance training, regardless of intensity and frequency.

The effect of exercise over a period of 3 years on stopping or decelerating of bone loss during the early postmenopausal years was assessed by Engelke et al. (2006). The application of the high-intensity exercise program succeeded to maintain bone mineral density at the spine, hip and calcaneus, but not at the forearm. Chow et al. (1987) evaluated the effect of 1-year aerobic and strength exercise programmes on bone mass. They found that both exercise groups showed a significant improvement in measured parameters. The effect of a 2-year exercise intervention and calcium supplementation (600 mg) on BMD was assessed by Kerr et al. (2001). Three groups of patients (strength, fitness, no exercise control) participated at this study. There was no difference between the groups at the forearm, lumbar spine, or whole body sites. The significant effect of the strength program was found at the hip (intertrochanter hip site). Judge et al. (2005) tested the effect of the resistance home-based training on the femoral BMD in long-term users of hormone therapy. The exercise decreased bone turnover and increased femur BMD. Korpelainen et al. (2006) didn't found the effect of long-term impact exercise on BMD at the radius and hip, while there was a positive effect on bone mineral content at the trochanter. High-impact loading exercise in osteopenic postmenopausal women was assessed by Chien et al. (2000) and Vainionpää et al. (2005). A 24-week program had a positive effect on the deceleration of the decline in BMD (Chien et al., 2000). Vainionpää et al. (2005) suggested that this type of exercise may be an efficient way to prevent osteoporosis. Martyn-St James and Carroll (2009) assessed the effects of mixed exercise programmes on postmenopausal bone loss at the hip and spine. The exercise programmes combining jogging with other low-impact loading activity and programmes mixing impact activity with high-magnitude exercise could be effective in reducing bone loss at the hip and spine. The effects of slow (strength) and fast (power) resistance exercises on various osteodensitometric parameters were compared by Von Stengel et al. (2005) and Von Stengel et al. (2007). The changes in BMD after 1 year of training were not significant for the power exercise group, whereas the BMD value in strength exercise group was significantly lower.

A comparison of the effect of exercise on BMD in postmenopausal women described in various studies is very difficult because of different number of subjects, exercise intensity, type of exercise, medication etc. In future research it will be necessary to exactly determine all factors involved and attempt to assess their influence on research results. Physical activity should be observed not only during exercise units but also during ordinary daily activities. However, results of most studies show that exercise has an important positive effect on the deceleration of decline in BMD.

5.1.2 Type, intensity and method of exercise

In order to evaluate the effect of physical activity on the change of bone markers, it is also important to take into consideration its parameters (type, intensity, duration …). In our case the exercise was of a lower intensity and was focused mainly on rehabilitation. And so the major effect did not lie in changing the physiology of the load but rather in improving body posture, adjusting muscle imbalances and postural stability.

Melendez-Ortega (2007) states that the amplitude of the load plays a more important role for bone density than number of repetitions. Repeated strain of bones above the physiological limit might lead to injury or even bone fracture. Englund et al. (2005) and other authors regard long-term weight-bearing training as the most suitable activity influencing the BMD. Vainionpää et al. (2005) proved that this type of exercise not only affects the bone density but also improves its architecture. To the contrary, Schwab and Klein (2008) claim that short repetitive loading of the bone has a positive impact on the biology of the bone.

Intensive aerobic activity, heavy-load and resistance exercises are, according to Yamazaki et al. (2004), much more effective in increasing BMD than physical activity with lower intensity (e.g. slow walking). Similar results were ascertained by Maddalozzo and Snow (2000) who believed that the highest effect is brought through a programme with high intensity of exercises. Heinonen et al. (1996) and Chien et al. (2000) determined that intensity of physical activity should hover above the aerobic threshold, i.e. above 60-70% of maximum aerobic capacity.

Brooke-Wavell et al. (2001), Yamazaki et al. (2004) and other authors recommend walking as a suitable activity for increasing bone density. Walking is the easiest and best available form of physical activity, which can be practiced virtually anywhere, poses only small risks of injury and requires negligible financial demands (it is, however, necessary to take into account risks of fall on uneven or slippery terrains). The most effective method of prevention of osteoporosis is brisk walking (Brooke-Wavell at al., 2001). Feskanich et al. (2002) point out, however, at increasing risks of fall at higher walking speed. Nevertheless, there exist studies which did not prove effect of walking on the increase of BMD (Martyn-St James & Carroll, 2008).

Exercise regimes encompassing a combination of weight-bearing, balance and coordination exercises but excluding jumping activities, improve BMD, enhance muscular strength and walking ability and thus reduce the risks of fall and suffering consequent fractures (Englund et al., 2005). According to Feskanich et al. (2002), activities improving balance and flexibility significantly contribute to reducing the risks of fall, whereas heavy-load and resistance exercises enhance muscular strength and BMD.

One of the shortcomings of the presented study is its limited scope of quantifying physical activity performed by women in the course of the monitored period. Common daily activities can have the same or even higher impact than directed exercise intervention. A survey

conducted within the study research, whose rate of return was, however, only approx. 25%, showed that in the run of one day, every woman had on average about 4.5 hours of physical activity (walking 1.2 hr; tidying up/cleaning 1.2 hr; meal preparation 1.1 hr; gardening 0.7 hr; shopping 0.3 hr).
Regrettably, these common everyday activities of monitored women are, in many studies, neither taken into account nor specified nor quantified. Most authors limit themselves to stating that the monitored applicants lead a sedentary type of life. It is, however, evident that such a definition of the level of everyday activities may not be sufficient.
Williams (1999) claims that women who never exercised can increase their BMD through physical training by 3-5% per year. Women who exercised regularly already have higher BMD and in order to maintain this level, they have to walk or run for 30 min a day, at least 5 times a week. The individual extent of physical activity in monitored patients can differ significantly with regards to the previous style of life. Kerry (2003) did not find any positive effect of physical activities related to house chores on the quality of bone tissue. On the other hand, most other researches do confirm the above-mentioned positive effect.

5.1.3 Medication

In the event that a patient does not suffer from any associated health disorder and is sufficiently hydrated, we do not expect significant changes in values of Ca, P and creatinine brought about by medications. Indicators of Ca and P do not have any direct relation to the level of bone remodelling (Štěpán et al., 2002). Changes in the values of osteocalcin and crosslaps express the activity of bone metabolism. In this case, a wide range of physiological values can be found, determining the standards in post-menopausal women (0.251-0.760 ng/ml resp. 4.9-30.5 ng/ml). It follows that it is necessary to evaluate measured changes in a strictly individual way. We have to keep this in mind also when evaluating statistically significant differences. Application of bisphosphonates (NEX/BP, EX/BP) is the most effective means for reduction of resorption expressed by the crosslaps parameter. Application of selective modulator (ralofixen; NEX/SERM, EX/SERM) influences these changes less and after a longer period of time. The decisive element is, however, a particular individuality of the given patient.
Some authors regard, as the most effective way to prevent loss of BMD, the combination of physical activity and hormonal treatment (Angin & Erden, 2009; Yamazaki et al., 2004). Specker (1996) claims that in order to create positive effect of physical activity on BMD, exercise must be complemented by application of calcium.
Results presented in this study confirm the opinion that exercise can in women after menopause minimize or inhibit the loss of BMD, it cannot, however, substitute pharmacological treatment and ensure increase in BMD (Bloomfield, 2005).

5.2 Exercise and balance

Risks of fall count among the most momentous problems in people with osteoporosis. Decrease of BMD of colli femoris in elderly people increases the risks of occurrence of fractures up to 2.6 times (Cummings et al., 1993). Awareness of the risks of fall can lead to intentional decrease in daily living activities, which is reflected in reduction of life quality. That is why it is essential to try and improve, using non-invasive interventions, the level of postural stability. One of the options bearing positive impact on improving balance control is regular physical activity.

Among interventions focused on reduction of the risks of fall counts, among others, training of balance keeping and walking (Messinger-Rapport & Thacker, 2003). For better stability, balance training is more effective than general exercise programmes including merely aerobic, weight-bearing or stretching activities (Rogers et al., 2003; Madureira et al., 2007).

In order to evaluate the stability in various modifications of stand, we used parameters of confidence ellipses created on the basis of COP movement. Differences in sizes of COP sway between both monitored groups are not significant. These findings differ from conclusions of other authors.

Effect of physical activity on improving balance and quality of postural stability is reported by Binder et al. (1994), Hopkins et al. (1990) and Gerdhem et al. (2003). Reducing COP sway after performing targeted exercises was noted by Perrin et al. (1999). Park et al. (2008) inquired into the effect of physical activity (48 weeks) on balance and compared postural sway between participants who did or did not do exercises. He found a significant difference in the COP sway in medio-lateral direction, whereas antero-posterior sway remained unchanged. Kuczyński & Ostrowska (2006) recorded the size of COP sway in medio-lateral direction higher by 50% in patients with osteoporosis than in healthy individuals. These authors also discovered that medio-lateral sways are higher in people who had fallen at least once in the past than in people without such history. Limitation or loss of lateral stability thus increases the probability of fall occurrence (Melzer et al., 2004). According to Hu and Woollacott (1994), multisensory training influences postural stability in a form of improving its parameters, among others, also when standing with head extended.

Differences in size of COP sway measured in our study, which are different from trends in the above-mentioned studies, can be caused by low demands of selected types of postures and by incoherence of the monitored group. The group was put together on the basis of diagnostics of osteoporosis or osteopenia, excluding women with neurological, cognitive and sensory disorders. When putting the groups together, associated disorders and occurrence of suffered injuries in the past were not taken into account. The monitored group represents just a small sample and the numbers of women in the exercising and non-exercising groups were not even.

Conclusions concerning deterioration of posture stability with closed eyes, which were ascertained for various groups of population, from few-month-old babies (Jouen, 1988) up to elderly people (Lord & Menz, 2000) were not confirmed. Influence of vision is further accentuated also in situations when the proprioceptive element of the stability control is reduced (Redfern et al., 2001).

Unlike the size of COP sways, effect of balance exercises was discovered for the speed of COP sway. In the stance with closed eyes and stance on foam, the exercising group significantly reduced speed of COP sway in comparison with the non-exercising group. Significant differences in reduction of the COP velocity in the exercising group were found also by Rogers et al. (2001).

Postural stability decreases with increasing asymmetry in distribution of physical weight (Genthon & Rougier, 2005). In our study, we did not find any significant differences between weight-bearing distribution on left and right legs in individual types of stands in any of the measured groups. Ascertained differences are significantly lower than the size

10%, which is considered as marginal for determining the asymmetry of weight-bearing (Véle, 1995).

5.3 Future research

Similar researches stated in literature do not pay sufficient attention to analysing daily living activities of patients. But in fact, the style of life of the monitored women can significantly influence obtained results. That is why it is necessary for any further research to evaluate physical activities performed above the scope of the targeted intervention. In order to determine their range and intensity, devices enabling quantification of these parameters should be employed.

In our research, the final measurements were conducted one year after the initial measurements, i.e. in the same season of the year. In the event of control measurements, which are conducted in shorter time intervals or under significantly different climatic conditions, it is important to determine the effect of these changes on the measured parameters.

Taking into account the level of motor skills and fitness of monitored people, it is necessary, when evaluating postural stability, to perform more demanding motor tasks (while abiding by all safety rules). When examining the effect of exercise, measuring dynamic stability could be more conclusive. Another option is to determine the level of stability when performing a specific task (dual task), whether motor or cognitive. Patients would then concentrate not only on their stance, but also on the performance of these tasks. Another means of achieving more reliable results could be a combination of exclusion or limitation of two sensory systems, as well as the modification of the visual signal with the use of special tools.

6. Conclusion

BMD in the L1-L4 area and colli femoris increased in all monitored groups (exercising as well as non-exercising). The change was caused by application of nonspecific (Ca+D3) and specific (Fosamax, Evista) pharmacological treatment. Increase was higher for the area of lumbar spine. Sizes of measured bone markers did not significantly change in the course of the monitored period.

A significant effect of exercise applied in the course of one year on the level of bone mineralisation was not confirmed. Thus, physical activity is a necessary requirement for positive changes of BMD in women after menopause, it is, however, not sufficient.

Effect of repeated exercise units focused on postural stability did not manifest in the sizes of the COP sway in a bipedal stance, however, it had an effect on decrease in the velocity changes of the COP positions.

In order to evaluate the postural stability in women with osteoporosis (osteopenia) without any significant limitations in the motor control area, it is essential to complement the execution of current tests with a simultaneous solution of other tasks.

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25

Impaired Ability to Perform the Sit-to-Stand Task in Osteoporotic Women

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1. Introduction

The process of aging causes several physiological alterations and body modification in elderly people. These changes include decrease in bone mass and muscular strength, rigidity in joints, and range of movement reduction in addition to changes in the central nervous system such as slow nerve conduction velocity (Deschenes, 2011), co- contraction of antagonist muscles, and alterations in the sensorial systems (visual, somatosensitive, vestibular functions), all contributing for the impairment of postural control and functional activities (Gauchard et al., 2003).

The maintenance of the postural stability is important during functional activities such as sitting down and standing up, walking, as well as for performing volitional movements co-ordinately, which is essential for daily tasks. The increase of postural control system deficit, which is associated to aging, has a strong relation to the risk of falls. In Brazil, data from the Ministry of Health shown that 28,459 elderly persons had died between 1979 and 1995 due to falls and, in February 2000, the inpatient mortality rate for falls was 2.58%.

Changes in postural control and decreased muscle strength and power are important risk factors for falls, especially in the elderly, since there is a reduction in muscle strength of 30-50% between 30 -80 years, especially in the lower limbs (Burke et al., 2010; Janssen et al., 2002).

The reduction in muscle strength occurs due to the reduction in the number and size of muscle fibers. Mainly type II fibers (fast contraction), which are most affected in relation to type I fibers (slow contraction) (Frontera et al., 1991).

The intensity of the muscle fibers loss depends on the degree of physical activity, nutrition, hereditary factor and lifestyle throughout life. In addition to losing in maximum muscle strength due to the aging process, there is also the loss of muscle power (force x speed), leading to a greater impairment in performance of functional activities, which requires agility, such as standing and walking, and increase susceptibility to falls (Marsh, 2000; Hunter et al., 2004).

The sit-to-stand test is a widely used tool in clinical practice because it is easy to apply and it requires simple matters, which are chair (without armrests) and a stopwatch. There are several ways to perform the test, but the way that is most commonly used, is to perform five

repetitions as quickly as possible, where the shorter the time to accomplish this task, the better the performance of the individual (Kim et al., 2010).
The risk of falls is increasing among elderly individuals who have difficulty in standing up from the chair (Campbell et al., 1989; Nevitt et al., 1989), since people with history of falls take more time to stand up from the chair and to stabilize their trunk after achieving the orthostatic position (Cheng et al., 2001). It is known that the action of standing up and sitting down is affected by decreased muscular strength and power, sensorial alterations, balance, and velocity in which this task is performed (Karikanta et al., 2005).
The incidence of falls increases with age and this fact is of great concern among the elderly, particularly among those with osteoporosis who present increased bone fragility, which increases the risk of fractures (Honig, 2010).
However, it is not clear if the osteoporotic women have a greater muscle function impairment compared to women with less bone losses and, consequently, have poorer dynamic postural control, which increases the risk of falls.
The muscle-bone unit has been suggested based on the mechanostatic theory, since muscle contractions promote tension in the bone, with consequent bone modeling activation. Therefore, the increase of muscle mass is also accompanied by increase of bone strength and improvement of bone geometrical characteristics (Hasegawa et al., 2001; Frost, 2003; Fricke & Schoenau, 2007). Also, the positive effect of some physical exercise on the increase of both muscle strength and bone mineral density (BMD) corroborates the relationship between bone and muscular systems.
Following these statements, the increased bone loss is accompanied by an increase of both muscle mass and muscle strength losses. However, based on the muscle-bone unit, the decrease of bone is consequence of both decrease of muscle mass and strength (Hamilton et al., 2010).
Therefore, in women with osteoporosis is expected an impairment during the functional activities performance, as sit-to-stand task, since they require the action of muscular system. The postural control impairment during functional tasks (standing up and sitting down) can make these individuals more susceptible to falls and subsequent fractures. For this reason, it is important to investigate factors that may worsen postural control in order to prevent falls, injuries and to improve the quality of life.

2. Objectives

To evaluate whether osteoporotic women have impaired dynamic balance in relation to those women presenting less bone loss by using the sit-to-stand test.

3. Hypothesis

Women with higher bone loss have greater impairment in postural control, needing to perform a greater flexion of the trunk to perform the sit-to-stand task, as a way of compensate for a probable weakness of the lower limb muscles, and spend more time to perform the test, compared to women with lower bone loss. The bone mineral density (BMD) loss can occur in association with a reduction of muscular capacity, due to the fact that muscular and skeletal systems act as a unique unit. The evaluation of dynamic balance through the sit-to-stand test can be an efficient method to detect the association between bone and muscular systems.

4. Methods

A cross-sectional study, which sixty women were divided into three groups according to the World Health Organization (WHO) classification of osteoporosis: Group 1 (n = 20) consisted of women presenting T score greater than - 1 standard deviation (normal bone mineral density), Group 2 (n = 20) consisted of women presenting T score ranging from -1 and -2.5 standard deviation (osteopenia or low bone mineral density), and Group 3 (n=20) consisted of women presenting T score lesser than -2.5 standard deviation (osteoporosis). For Group 1, the mean age was 65.75 years (± 4.33), mean weight was 64.81 kg (± 6.83), and mean height was 157.0 cm (± 6.0). In Group 2, the mean age was 67.45 years (± 4.57), mean weight was 62.63 kg (± 10.21) and mean height was 156.0 cm (± 7.0). In Group 3, the mean age was 70.0 years (± 5.43), mean weight was 68.97 kg (± 15.01) and mean height was 155.0 cm (± 8.0).

Women presenting vertebral fractures diagnosed by radiographs, diabetes mellitus, peripheral neuropathies, cardiovascular diseases, vestibulopathies, and neurological problems were excluded from this study. All women were sedentary, no smoker and none of them was included in any kind of rehabilitation program.

The participants were selected from the general community and from the Centre of Health at the Ribeirao Preto School of Medicine, FMRP-USP (CSE-FMRP-USP) and affiliated Clinic Hospital. The research study was approved by the Human Research Ethics Committee of the Ribeirao Preto School of Medicine, University of São Paulo (protocol number 1953/2007), with all the volunteers signing a free informed consent before participating in the study.

All the participants were submitted to evaluation of dynamic activity using the Polhemus system, in which the maximum antero-posterior dislocation of the trunk and time spent for practicing the test were evaluated.

The Polhemus system (POLHEMS ® 3 SPACE ISOTRAK II, Conchester, Canada) (Abreu et al.; 2010) was employed for evaluation, which is based on emission and detection of magnetic fields by means of electromagnetic sensors (Figure 1). The emission system comprises three perpendicular coils (55 x 55 x 58 mm) and the detection system is also formed by other three perpendicular coils (2.9 x 28.3 x 15.2 mm), and an amplifier was used to obtain x (antero-posterior), y (medio-lateral), and z (vertical) orientations. The transmitting coil was positioned onto a support that was placed at 60 cm from the subject, whereas the sensory coil was fixed to the seventh cervical vertebra (C7). The equipment precisely measures every trunk movement: the attached transmitting coil emits magnetic fields which are detected by the sensor, considering that the distance between the transmitting and sensory coils is known.

The dynamic activity was evaluated during the sit-to-stand (STS) (Bohannon, 2006) movements five times so that the maximum trunk antero-posterior dislocation and time spent for practicing the test could be obtained. An armless chair with seat height of 43 cm was used for this test. The subject started the test with her both feet on the floor and her arms crossed at the chest level. Then she was asked to perform the sit-to-stand movements five times consecutively as quickly as possible, and the test was concluded when the evaluator asked the subject to remain in the chair (Figure 2).

Analysis of variance (ANOVA) was used for comparison between the groups. According to these statistical models, the residual differences between predicted and measured values have a normal distribution, with zero mean and constant variance. In the situations where such a presumption was not observed, changes in the response were taken into account. Post-hoc Tukey test were performed when needed. This procedure was performed by using the SPSS 16.0.

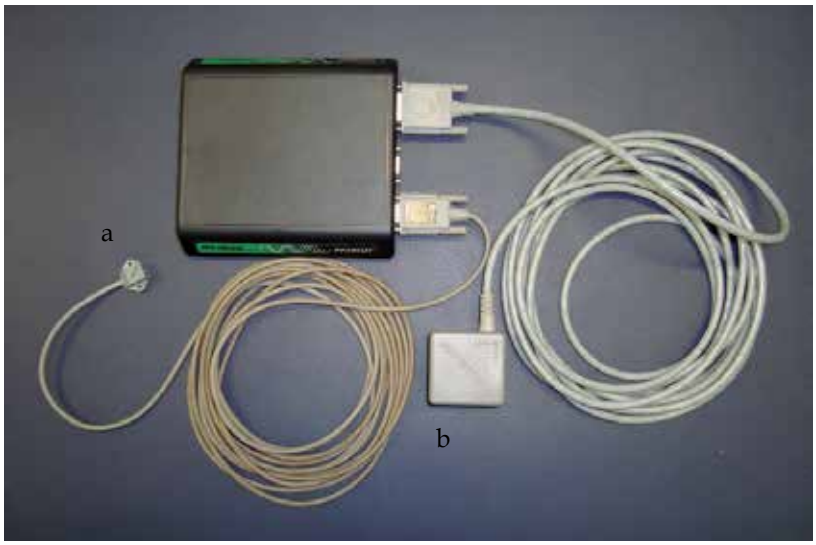


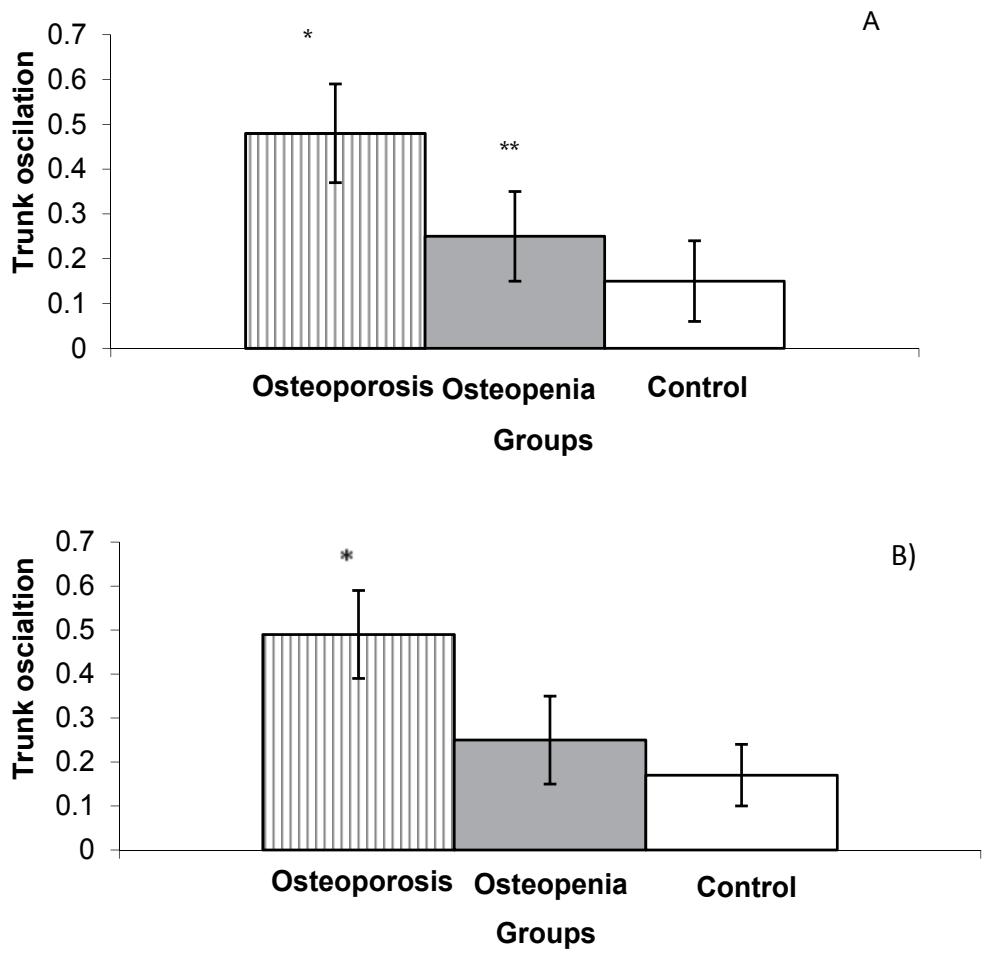
Fig. 1. Illustration of the Polhemus system. a) sensor coil; b) transmitting coil.

Fig. 2. Illustration of initial position for the sit-to-stand test. Arrows indicate the positions of sensor coil on the seventh cervical vertebra and the transmitting coil.

5. Results

The results showed that variables such as weight and height showed no significant differences between the groups ($P > 0.05$), but age was found to be different between the control and osteoporotic groups ($P < 0.01$). For this reason, the variables maximum antero-posterior dislocation (cm) during standing up and sitting down tasks and time (seconds) spent for practicing the test were normalized by age.

Post-hoc Tukey test showed that women with T score lesser than - 2.5 SD (osteoporosis) had greater movement of the trunk during standing up and sitting down tasks compared to other groups of women. Also, women with T score between -1 and - 2.5 SD had greater movement of the trunk in relation to the group of women presenting normal bone mineral density (T score greater than – 1 SD) during stand up from the chair (Figure 3).



*P < 0.05 osteoporosis versus osteopenia and control
** P < 0.05 osteopenia versus control

Fig. 3. Antero-posterior dislocation (cm) during the standing up (A) and sitting down (B) tasks. Values were normalized by age.

The results showed that there were no differences in the time spent to perform the sit-to-stand movements ($P > 0.05$) between the groups (Figure 4).
In the Group 3 (women with osteoporosis), the time spent for performing the STS test was 15.14 ± 4.18 sec (0.22 ± 0.06 sec after normalized by age) and the antero-posterior movements of the trunk during standing up and sitting down tasks were, respectively, 33.03 ± 7.04 cm (0.48 ± 0.11 cm after normalized by age) and 34.15 ± 6.27 cm (0.49 ± 0.10 cm after normalized by age). In Group 2 (women with osteopenia), the overall time spent was 13.34 ± 3.34 sec (0.20 ± 0.07 sec after normalized by age), with antero-posterior movements of the trunk during standing up and sitting down tasks being, respectively, 16.98 ± 11.03 cm (0.25 ± 0.17 cm after normalized by age) and 16.61 ± 11.61 cm (0.25 ± 0.17 after normalized by age). With regard to Group 1 (women with normal BMD), the time spent for performing the STS test was 11.95 ± 2.1 sec (0.18 ± 0.03 sec after normalized by age) and the antero-posterior movements of the trunk were 9.86 ± 9.12 cm (0.15 ± 0.13 after normalized by age) and 10.96 ± 9.74 cm (0.17 ± 0.16 after normalized by age) during standing up and sitting down tasks, respectively.

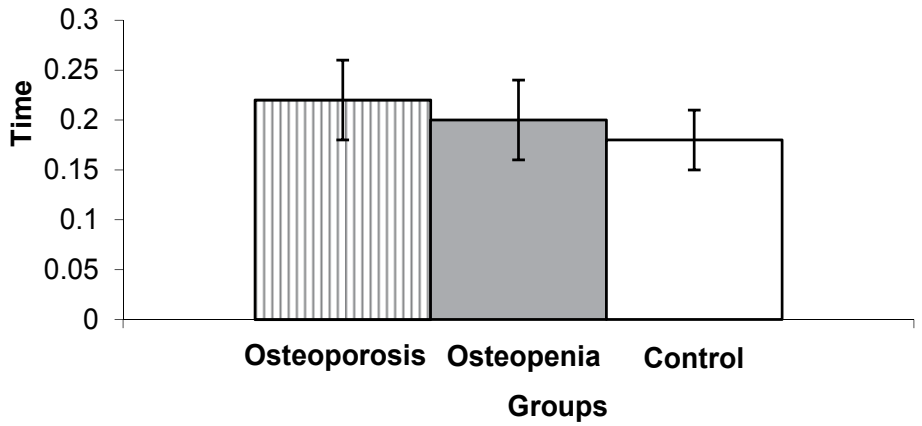


Fig. 4. Time spent (sec) during the sit-to-stand task. Values were normalized by age.

6. Discussion

The aging process is associated to many changes of body function, which include alterations in the postural control during posture maintenance and activities, which interfere directly with balance and lead to an increase in corporal oscillation in elderly persons (Gill et al., 2001).
The postural control system requires the association of perception (of body position and body movement) (Faraldo-García et al., 2011), action (capacity of muscle activation) and cognition (movement planning and execution). In order to have an efficient action it is necessary, besides muscle function, other biomechanical aspects, as adequate range of movement and posture. The muscle deficits can have a negative influence on postural control and functionality, because they decrease the ability to perform functional movement with safety and efficiency which increase the risk of falls (Shumway-Cook &, Woollacott, 2000). Additionally, the decrease of muscle strength and power in elderly persons has a negative impact on the ability to restore the state of balance after external perturbations.

The sit-to-stand (STS) test is easy to apply and it is one of the most used methods to assess the functional muscle strength as well as the balance and the functional mobility of elderly people (Bohannon, 2006; Buatois et al., 2006), thus allowing identification of those individuals with impaired balance (Whitney et al., 2005). In a study carried out by Aslan et al., 2008, who applied the sit-to-stand test to young and older adults, the results showed that elderly individuals spent more time to perform the tasks compared to the young ones. Zech et al., 2011 also shown that the sit-to-stand power test can be efficient to distingue between nonfrail elderly and prefrail. In elderly women with 60 years and older, both higher muscle mass and lower body fat were positively associated with physical function, evaluated by walking speed and sit-to-stand test (Visser et al., 2000).

The five-repetition sit-to-stand test has been used to evaluate muscle strength and balance. However, the action of sitting and standing involves a complex integration of muscular strength and muscle power, range of movement, postural control and coordination pattern (Karikanta et al., 2005). Some studies shown that the muscle contraction speed is very important to perform the sit-to-stand test, as fast as possible, and the weak power probably contributes to loss of mobility and it can better predict falls than muscle strength analysis (Skelton et al., 2002; Petrella et al., 2005). Also, the sit-to-stand test is adequate to evaluate muscle power in elderly population (Zech et al., 2011).

In a study conducted by Netz et al., 2004, they observed that the sit-to-stand test when performed 10 times consecutively, as fast as possible, is not able to predict knee extensor strength, but it can be used to predict general endurance. Also, they suggest that the peak aerobic capacity is related to the performance of the test. However, the sit-to-stand test 10 times is a longer activity that increase the chance of both muscle fatigue and change of coordination pattern, and possibly requires more of the cardiorespiratory system. Therefore, future studies must be performed to compare the methodologies (five versus ten times sit-to-stand test).

Nevertheless, it is not clear if the reduction of both muscle function and postural stability is more pronounced in women with osteoporosis. The impairment on the ability to perform functional tasks (for example: standing up and sitting down) can make these individuals more susceptible to falls and subsequent fractures. The rate of falls among the elderly is high and the fractures occurrence and severe lesions lead to a partial or total decrease in their daily activity performance and autonomy, with a negative impact in their quality of life (Shimada et al., 2003).

Therefore, in order to evaluate the association between bone mineral density and dynamic balance of elderly women, the sit-to-stand test was used, since it seems to be a clinical test capable of evaluating muscle function and balance. The variables obtained were maximum antero-posterior trunk movement during the sit-to-stand tasks and time spent during the test.

The data shown that women with osteoporosis had greater movement of the trunk during standing up and sitting down tasks compared to other groups of women. The results suggest that the decrease in BMD can occur in association with a reduction in the functional capacity of muscular system, due to the fact that muscular and skeletal systems act as a unique unit. A previous study shown that osteoporotic women are more likely to fall than the non-osteoporotic ones within the same age group, due to the fact that osteoporotic women have greater weakness of the quadriceps and impaired postural control (Lynn et al, 1997). The quadriceps muscle is important for performing the sitting down/standing up movement, which might explain why the group of osteoporotic had a greater anterior-

posterior movement of the trunk as a way of compensating for such a muscle weakness (Bohannon, 2006; Lord et al.; 2002).

Moreover, the women with T score between -1 and - 2.5 SD (osteopenia) had greater movement of the trunk in relation to the group of women presenting normal bone mineral density (T score greater than – 1 SD) during stand up from the chair. This finding is very relevant, since it shown that women with osteopenia already perform compensation during the dynamic activity. The fact of women with osteoporosis have greater movement of the trunk during standing up and sitting down suggests a quadriceps function deficit during concentric and eccentric contractions, while the fact that women with osteopenia have greater movement of the trunk only during standing up suggests some quadriceps muscle function deficit only during concentric contraction.

Based on the literature, the aging process affect negatively all contraction muscle types (concentric, eccentric and isometric strengths) (Lindle et al., 1997; Porter et al., 1995), however, the concentric knee extension strength decreases more than eccentric strength. Therefore, it seems that women with osteoporosis have a more pronounced decline of muscle contraction strength, with impairment during concentric and eccentric movements.

The compensatory movement by the trunk dislocation is worrying, since the high degrees of trunk flexion movement displaces the centre of gravity anteriorly, and associated to the decrease of postural stability and muscle function, the control of body mass centre is prejudiced, resulting in difficulty to maintain the state of balance during the sit-to-stand task, which increases the risk of fall among the osteoporotic women.

There was no difference between groups when compared the time spent during the sit-to-stand movements ($P > 0.05$), probably due to the higher trunk flexion performed by groups with osteoporosis and osteopenia, which compensate the lower limb muscles deficit and, consequently, allowed them to achieve the same time during the test. However, in some studies in which the sit-to-stand test was applied to elderly individuals, the results regarding the time spent for performing the tasks are close to those obtained in the present study (Whitney et al., 2005; Aslan et al., 2008; Schaubert & Bohannon, 2005), which suggests that the decrease of the movement velocity is associated to many factors of aging process and not to the bone mineral density (BMD) directly.

The spent time to perform the five-chair sit-to-stand test in women aged 65 years or older with osteopenia (Chyu et al., 2010; Alp et al., 2007) was similar to the present study. In another study conducted with osteoporotic women (Alp et al., 2007), the sit-to-stand test was performed 10 times as quickly as possible, and the values obtained was approximately twice the time spent by women in our study (in our study they performed the sit-to-stand movements five times consecutively as quickly as possible).

Lindsey et al, 2005 did not observe a correlation between sit-to-stand test performance and BMD of any skeletal site in older women, which is in agreement with our findings, since we did not find differences in time spent during the sit-to-stand test between groups. Also, in a systematic literature review conducted by Hyehyung et al., 2011, no correlation was observed between lower femoral/lumbar BMD and slower sit-to-stand test in age-adjusted models.

The obtained results point out an interesting discussion on which parameters should be considered in assessments of dynamic balance and functional activity, since the compensatory movements can mask the deficits of analyzed variables. In our study, the antero-posterior trunk dislocation probably was the compensatory movement due to the lower limbs muscle weakness. The lack of difference in the spent time during the sit-to-stand test is probably a consequence of the compensatory movement. This aspect has

already been raised by Netz et al., 2004, since they discussed that the STS ten times not include the trunk control analysis.

Hence, the evaluation of spent time to perform the STS test by itself would not be enough to identify impairment in the dynamic activity in women with greater bone losses and wrong conclusions about the muscle strength and balance characteristics could have been done.

In relation to the muscular system, the aging process interferes negatively in muscle characteristics (Zech et al., 2011; Clark BC & Taylor, 2011; Buffa et al., 2011), and following the muscle-bone unit theory, women with greater bone losses also present greater muscle function alterations. Therefore, the compensatory movement (increase of the trunk dislocation) observed during the sit-to-stand test can also be associated to a decrease of muscle function, which includes strength, power and muscle mass, without disconsidering the other components involved during the test. However, future research is needed to verify the muscle function in women with different levels of bone mineral density.

Our hypothesis was in part confirmed, since women with different classifications of BMD presented different anterior-posterior displacements of the trunk, but not presented differences of time spent to perform the sit-to-stand test.

Besides, the results show that women with osteopenia also have increase in the trunk dislocation, and based on a study by Siris et al, 2001 which shown that osteopenic women had 1.8-fold higher rate of fracture than women with normal BMD, a careful attention should be paid to this population in order to reduce the risk of falls.

A recent study evaluated the physical performance of women aged 45 to 64 years, through evaluations that included sit-to-stand test (Khazzani et al., 2009). The results showed that low physical performance was associated with low BMD of spine and hip. In addition, some studies shown that regular training to strengthen the muscles of the lower limbs, especially the quadriceps, are effective for increasing muscle power, static and dynamic balance, thus improving performance activities of daily living, which includes the act of sitting down and up (Khazzani et al., 2009; Teixeira et al., 2010). Also, high-impact loading exercise has shown to be efficient to increase bone mass and geometry in postmenopausal women (Hamilton et al., 2010; Iwamoto et al., 2010). Another study that conducted a 11-month exercise program, which included strength, aerobic capacity, balance, joint mobility on ground and in the water on postmenopausal women shown an improvement of physical function capacity, associated to a reduction of physiological bone loss (Tolomio et al., 2010).

Those studies ratify the importance of exercise programs in order to improve muscular and bone systems and to improve balance and functional capacity. The conservation of muscle function seems to be essential to keep a sufficient mechanical stimulus on bone, and consequently, to minimize the bone decline over time.

These data emphasize the need to encourage women with different levels of bone loss to adhere to exercise programs in order to improve their balance, functionality and bone characteristics, thus reducing the risk of falls and fractures consequently.

7. Conclusions

The results suggest that osteoporotic women exhibit a greater trunk movement compared to women with less bone loss, which is associated to impairment of both postural control and muscle function. However, women with T score ranging from -1 to - 2.5 SD (low bone mineral density) had a greater impairment compared to the group of women with T score greater than -1 SD.

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Osteoporosis and Arterial Stiffness: Effects of Exercise Training

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1. Introduction

In addition to menopause and advanced age, risk factors for atherosclerosis are also associated with osteoporosis. Osteoporosis and atherosclerosis are major public health problems that lead to increased rates of morbidity and mortality. Because these diseases progress with aging and share common risk factors, both seem to correlate with aging. Although historically considered as independent conditions, clinical and epidemiological studies indicate that common pathophysiological mechanisms underlie these diseases. Physical activity is of primary importance to reach optimal peak bone mass and decrease arterial stiffness, an independent risk factor of atherosclerosis. Exercising that incorporates levels of whole body accelerations exceeding 3.9 g at a frequency of 100 per day has been shown to have positive effects on cardiovascular fitness, femoral bone density and balance (Jämsä et al, 2006; Vainionpää et al, 2006; Heikkinen et al, 2007). These acceleration levels are normally reported in activities such as running or jumping, which may be appropriate for middle aged and younger individuals, but may be more difficult for many older people or those with chronic lower limb injuries to achieve. This chapter explains the effect of exercise on osteoporosis and arterial stiffness.

2. Arterial structure

Arteries are flexible, muscular blood vessels that carry blood from the heart and oxygenated blood to tissues throughout the body (Murray, TD. & Murray JM. 1998). The arterial wall comprises three layers (Fig. 1). The outermost adventitia primarily consists of connective tissue made of collagen, a structural protein that helps to maintain vessel integrity and provide flexibility. The elastin media is the middle layer, which mostly comprises smooth muscle tissue that confers the ability to contract and relax. This helps to regulate the size of the vessel lumen and thus alter blood pressure and flow. The inner intima layer comprises smooth epithelial tissue that facilitates blood flow. This layer includes the endothelium, which is the inner arterial wall.

3. Collagen and elastin on bone and arteries

About 80% of the total protein in bone consists of collagen, about 95% of which is type I. Bone strength depends on the orientation of osteons (and thus collagen fibers) within

cortical bone. Various determinants of bone quality are interrelated, especially minerals and collagen (Viguet-Carrin et al, 2007).

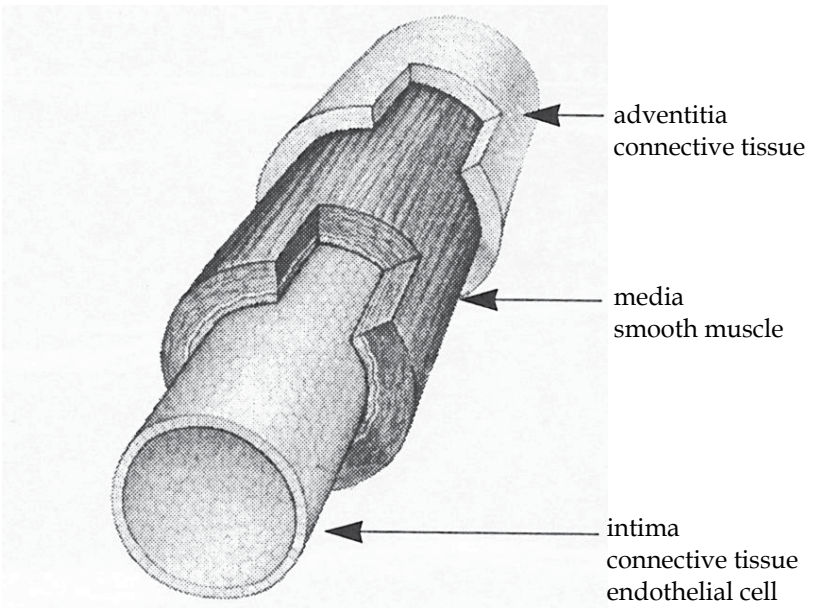

Fig. 1. Arterial structure

Collagen and elastin are two vital components of blood vessels (Greenwald, 2007). Elastic arterial fibers comprise 90% elastin, which enables tissues to resume shape after stretching or contraction (Milewicz et al, 2000). Collagen is the most common protein in mammals (25% to 35% of total body protein content) as it is the main component of connective tissue. Elastin and collagen play crucial roles in arterial remodeling. Moreover, arterial stiffness depends upon the composition of the elastin and collagen, and the calcium content of elastin. As collagen ages, specific physical and biochemical changes reduce extensibility and increase rigidity. Thus, aging increases the diameter of collagen fibers in various tissues. Fibrils also become more crystalline, which strengthens intermolecular bonds and increases resistance to further deformation. Furthermore, aging is believed to be associated with an increased number of intramolecular and intermolecular cross-links that restrict the ability of collagen molecules to glide past each other. Collagen fibers are only slightly extensible but are very resistant to tensile stress. Therefore, they are the main constituents of structures such as ligaments, tendons and arteries that are subjected to pulling forces. As a result of aging, elastic fibers lose resilience and undergo various other changes, including fragmentation, fraying, classification and other types of mineralization and increased cross-linkages (Knott et al. 1997).

4. Osteoporosis and arterial stiffness

The multifactorial and degenerative entities of osteoporosis and atherosclerosis are major public health problems. These diseases accompany the aging process and share common risk factors. Increased arterial stiffness independently predicts cardiovascular and

cerebrovascular events in healthy populations. Several studies have examined associations between atherosclerosis at different sites and osteoporosis or low bone mineral density (BMD) in women, and the findings suggest that the development of osteoporosis is a risk for advanced atherosclerosis after menopause (Hak et al, 2000; Sanada et al, 2004). The Osteo Sono-Assessment Index, which reflects elastic properties of bone tissues, negatively correlates with pulse wave velocity (PWV) in both sexes; this association is more prominent in females than in males and becomes even closer in post-menopausal females (Hirose et al, 2003, Fig. 2).

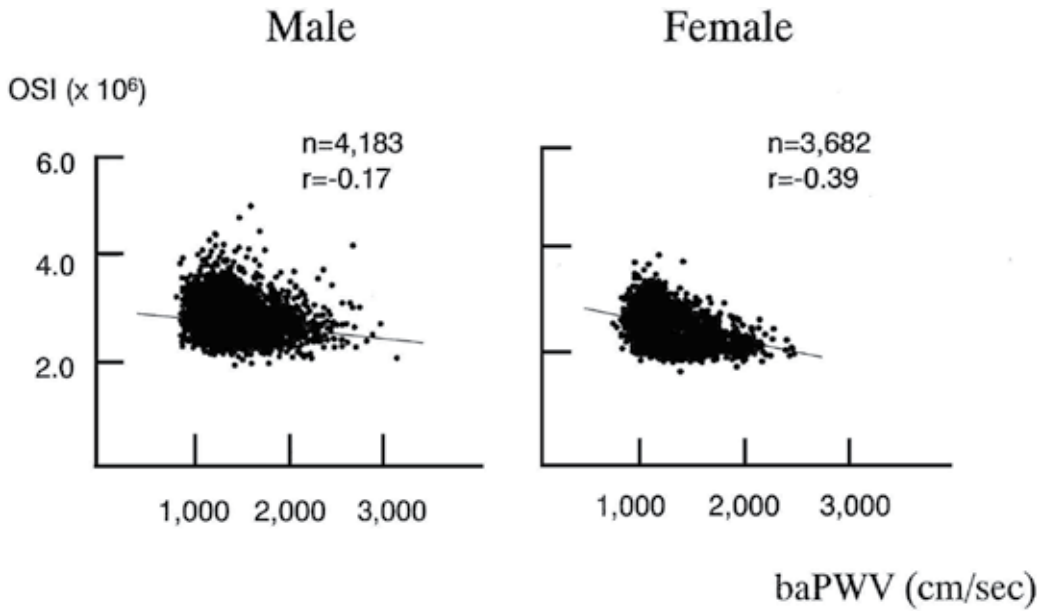

Fig. 2. Correlation between osteo-sono assessment index (OSI) and brachial-ankle pulse wave velocity (baPWV) in both genders (Quotation from Hirose et al, 2003).

Increased central arterial stiffness reduces the arterial buffering function of the pulsation of blood pressure and blood flow, which contributes to increases in systolic blood pressure and in pulse pressure. Increased arterial stiffness alters the cyclical dynamics of arterial wall connective tissues, promotes vascular remodeling, and increases arterial wall thickness and plaque formation. Patients with osteoporosis have the most arterial stiffness. The reciprocal association between osteoporosis and arterial stiffness is supported by the relationship between bone mineral loss and each of vascular calcification, atherosclerosis and cardiovascular disease (CVD). Arterial calcification leading to increased arterial stiffness, a powerful risk factor for CVD, might underlie the association between osteoporosis and CVD in post-menopausal women. Osteoprotegerin might be a molecular link between bone loss and vascular calcification. In fact, intimal calcification is associated with advanced atherosclerosis. In addition, Frost et al. (2008) suggested that decreased BMD is associated with arterial calcification and stiffening and raised the possibility that osteoprotegerin is a marker of arterial stiffening, independently of any association with BMD. Osteoporotic postmenopausal women free of CVD and risk factors had increased augmentation index, a measure of wave reflections and arterial stiffness, and central aortic systolic and pulse

pressures, which show a higher estimated aortic PWV indicating a stiffer aorta (Mangiafico et al, 2008, Tab. 1). Such alterations may increase the risk of CVD in postmenopausal osteoporosis. Therefore, the prevention and treatment of increased arterial stiffness and/or osteoporosis are important.

Parameter	Patients n=182	Controls n=160	P value
Brachial SBP (mmHg)	123.7±11.8	122.2±12.3	0.17
Brachial DBP (mmHg)	75.8±8.5	74.2±7.4	0.12
Brachial PP (mmHg)	47.9±11.4	48.0±10.8	0.77
Aortic SBP (mmHg)	117.5±12.1	111.4±12.2	<0.0001
Aortic DBP (mmHg)	76.9±8.4	74.9±8.0	0.28
Aortic PP (mmHg)	40.5±10.3	36.4±8.1	0.0007
Heart rate (beats/minutes)	71.9±7.6	73.6±12.9	0.80
Ejection duration (ms)	317.3±29.9	321.0±24.4	0.58
Augmentation (mmHg)	15.3±5.4	11.0±3.7	<0.0001
Augmentation index (%)	37.2±7.0	29.6±9.2	<0.0001
Timing of reflected wave (ms)	122.0±11.5	130.6±13.6	<0.0001
Subendocardial viability ratio (%)	134.6±14.5	134.9±30.5	0.28

SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; ms = milliseconds.

Table 1. Peripheral and central haemodynamic parameters of osteoporotic patients and control subjects (Quotation from Mangiafico et al, 2008)

5. Effects of aging on osteoporosis and arterial stiffness

In addition to menopause and advanced age, risk factors for CVD such as obesity and diabetes are also associated with osteoporosis. Thus, osteoporosis and atherosclerosis seem to correlate with aging. Osteoporosis-related fractures represent a major health concern, particularly among elderly populations. Post-menopausal osteoporosis in women with increased availability of circulating osteoprogenitor cells has a detrimental influence on arterial compliance. Lifestyle modification includes measures to reduce falls and bone loss such as participating in exercise, adequate dietary calcium intake and avoiding smoking and excessive alcohol consumption. Osteoporosis is characterized by the progressive loss of bone tissue and micro-architectural deterioration that reduces the quality of life for the elderly and thus it is a persistent public health issue. BMD at the femoral neck and spine in aging women decreases by 1 - 2 % per year (Finkelstein et al, 2008). Decreasing estrogen concentrations after menopause can cause a decline in BMD, which leads to osteoporosis. Epidemiological data suggest that estrogen deficiency is a risk factor for CVD and osteoporosis.

Changes in arterial function with age include a decrease in major artery compliance and increased arterial stiffness will result in an increase in resting and exercise blood pressure. Large arteries that convey blood at high pressure have relatively thick walls. Arterial stiffness, an independent risk factor for CVD, increases with advancing age (Tomiyama et al, 2003, Fig. 3). This age-related increase is greater in post-menopausal women, which increases their vulnerability to CVD. The cause of progressive age-related stiffness is the obviously increased thickness of the artery walls and interstitial collagen. Vessel structure also changes when an increase in blood pressure augments vascular tension. Increased arterial stiffness might be due to age-associated structural changes in the arterial walls. Aging is associated with a decrease in elastin and a concomitant increase in collagen and connective tissues in the arterial walls and an increase in arterial stiffness due to menopause.

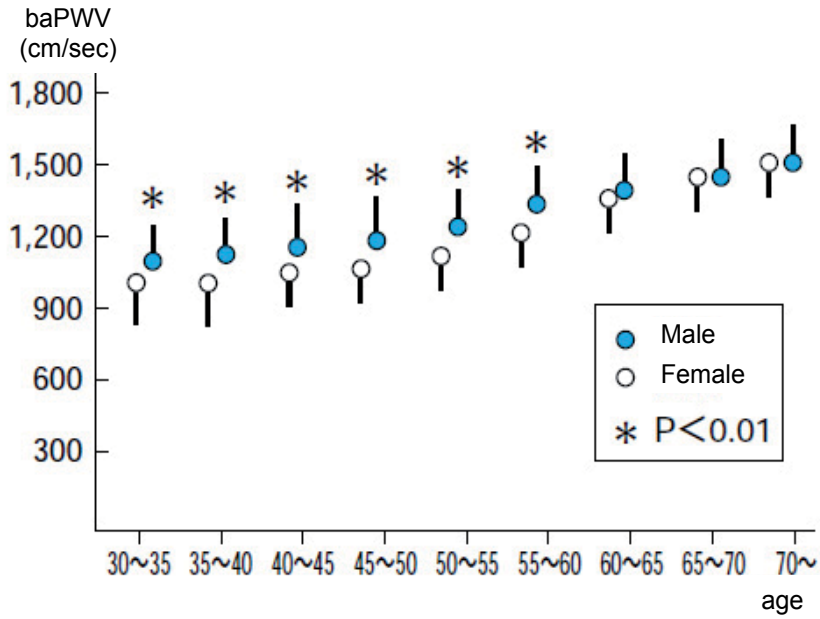

Fig. 3. Chronological changes in brachial-ankle pulse wave velocity (baPWV) in healthy men and women (Quotation from Tomiyama et al, 2003).

6. Exercise

Health organizations such as the American Heart Association (AHA) and the American College of Sports Medicine (ACSM) recommend habitual exercise to prevent and treat CVD and frailty associated with aging. In contrast to age, regular physical exercise in general, and aerobic exercise/fitness in particular, are associated with enhanced vascular function and a reduced risk of CVD. However, in contrast to the beneficial effects of aerobic exercise, high-intensity resistance training increases arterial stiffness in young and middle-aged healthy men and in pre-menopausal women.

To date, the predominant medical strategies to prevent and/or treat post-menopausal bone loss have focused on antiresorptive medications (i.e., bisphosphonates). However, these treatments might be limited due to adverse side effects, questionable compliance and long-term safety concerns. Various types of exercise, such as walking, jogging or resistance training, could provide an important role in maintaining and/or increasing bone density in women. Therefore, implementing non-pharmacological treatment strategies such as exercise that have few or no inherent side effects is critical. Exercise plays an important role in maintaining or increasing bone density. Physical activity increases growth in the width and mineral content of bones in girls and adolescent females, particularly when initiated before puberty, carried out in volumes and at intensities seen in athletes, and accompanied by adequate caloric and calcium intake. The differences are regularly the largest in gymnasts whose hip and spine BMD values are 30% – 40% higher than those of long-distance runners (Robinson et al, 1995); a plausible explanation for this is the greater magnitude of impact forces generated in gymnastic movements (10- to 12-fold body weight) compared with

running (3- to 5-fold body weight) (Duncan et al, 2002). Moreover, not only are high-impact sports associated with a greater BMD, but athletes involved in high-impact sports also have a greater section modulus (a predictor of strength in bending) (Nikander et al, 2005, Fig. 4). Since the two mechanisms that principally determine adult bone health are peak BMD at skeletal maturity and the rate of bone loss with advancing age, maximizing pre-menopausal BMD is a critical strategy for preventing osteoporosis and resultant fractures later in life.

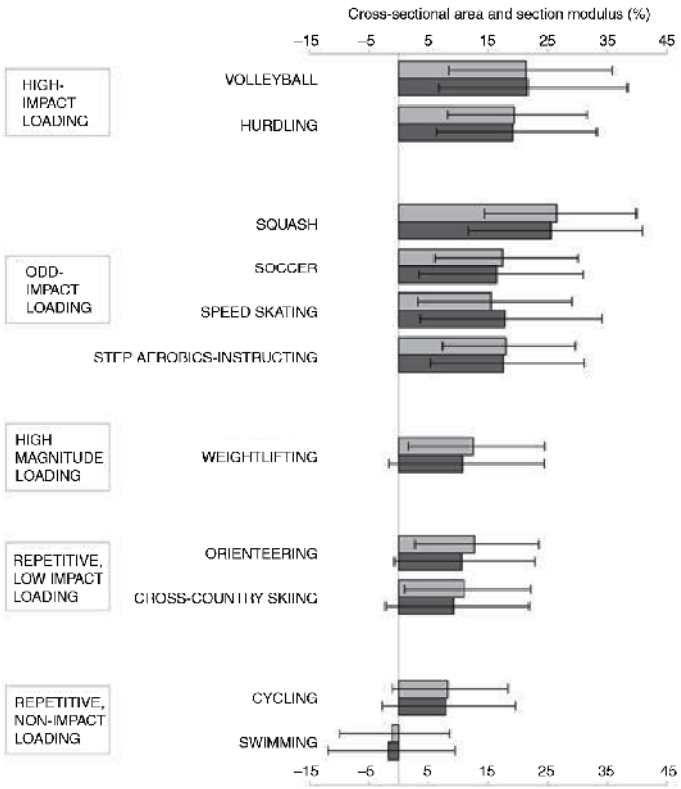

Fig. 4. Differences in cross-sectional area ( ) and section modulus (a predictor of strength in bending;) between athletes participating in sports of different loading modalities and controls. Values are means and 95% confidence interval (CI) represented by horizontal bars. Where the 95% CI does not cross the zero line (the value for the controls) the difference was significant (P<0·05) (Quotation from Nikander et al, 2005) .

6.1 Aerobic exercise and arterial stiffness

Physical activity can be used as a prophylactic tool against osteoporosis and to improve skeletal resistance to bone fractures. A physically active lifestyle is associated with a 30% to 50% decrease in the risk of vertebral or hip fractures. Aerobic exercise positively affects blood pressure and arterial stiffness. Regular aerobic exercise is recommended to prevent and treat CVD and the frailty associated with aging. Regular aerobic exercise is beneficial for reversing arterial stiffening in middle-aged and older adults (Tanaka et al, 2000, Fig. 5). Moderate, short-term aerobic exercise could restore carotid arterial compliance in previously sedentary post-menopausal women taking hormone replacement therapy (Moreau et al, 2002).

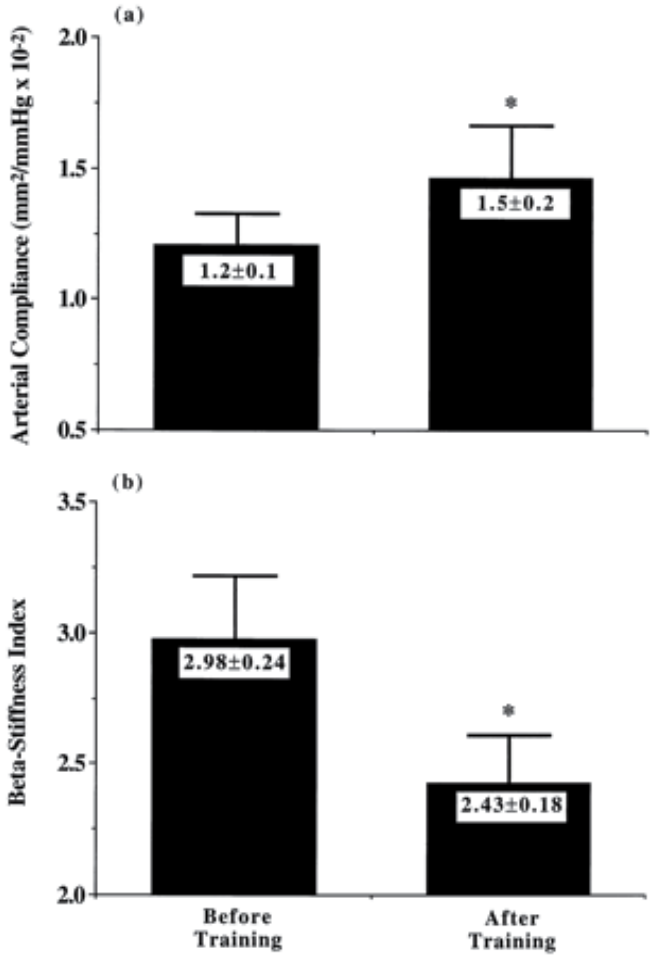

Fig. 5. Arterial compliance (a) and ß-stiffness index (b) before and after aerobic exercise intervention. *P<0.01 vs before training. (Quotation from Tanaka et al, 2000)

The ACSM position on physical activity and bone health recommends regular weight-bearing endurance activities, including jogging and jumping to preserving bone mass during adulthood. Moreover, although vascular function is not improved by aerobic exercise before resistance training, aerobic exercise thereafter can prevent vascular function from deterioratin (Okamoto et al. 2007, Fig. 6). Adaptive bone responses might require dynamic, rather than static mechanical stimulation. Aerobic exercise combined with high-impact exercise training seems to be effective against osteoporosis and/or for improving vascular health.

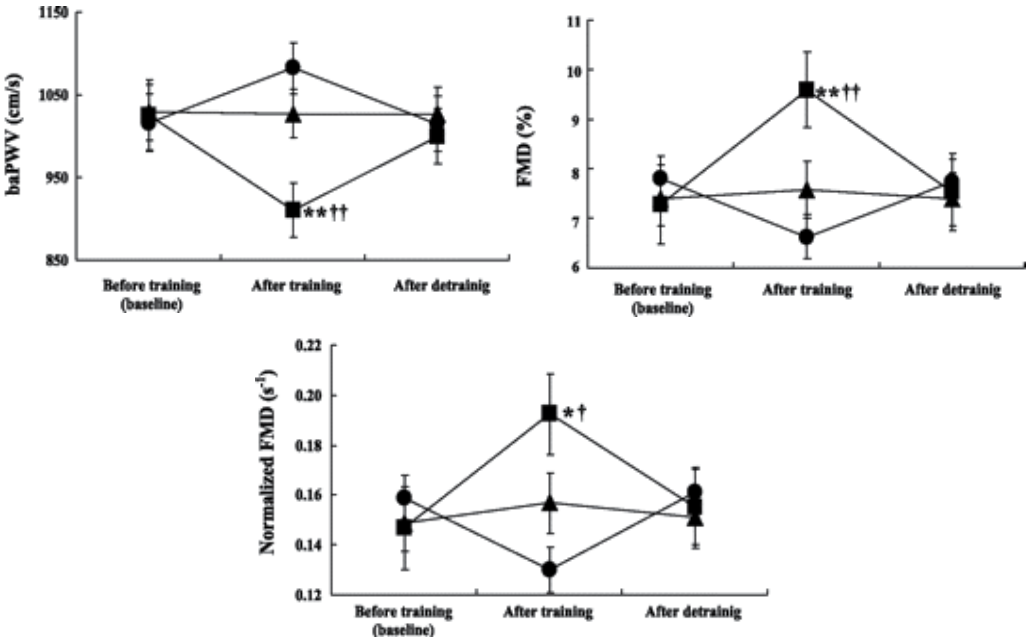

Fig. 6. Changes in brachial-ankle pulse wave velocity (baPWV), percent flow-mediated dilation (%FMD), and normalized FMD in groups that ran before resistance training (RT) (BRT;●), ran after RT (ART;■), or remained sedentary (SED;▲). Values are means ± SE. *P < 0.05; **P < 0.01 vs. baseline. †P < 0.05; ††P < 0.01 vs. BRT group (Quotation from Okamoto et al. 2007).

6.2 Resistance exercise and arterial stiffness

Physical activity could increase bone strength by increasing muscle mass (Bennell et al, 2000). Physical activity reduces skeletal fragility and a predisposition to falling through a combination of increased BMD and improved coordination, balance, reaction time and muscle function (Liu-Ambrose et al, 2004). Resistance training is a critical component in exercise prescription programmes for healthy adults. Resistance training is widely recommended to prevent sarcopenia and osteoporosis (Pollock et al, 2000). Resistance exercise at high intensity [one repetition maximum (1RM), 80%] has generally been regarded as optimal for gaining muscular size and strength (McDonagh, & Davies, 1984). However, high intensity resistance training has been associated with the stiffening of large arteries in young and middle-aged adults (Miyachi et al. 2004, Fig. 7). In contrast, Cortez-Cooper et al (2008) reported that 13 weeks of moderate-intensity resistance training two or three times per week does not reduce central arterial compliance in middle-aged and older adults. In addition, Yoshizawa et al (2009) demonstrated that 12 weeks of moderate-intensity resistance training did not affect arterial stiffness in middle-aged women. Moreover, low-intensity resistance training with short inter-set rest periods reduces arterial stiffness and improves vascular endothelial function (Okamoto et al, 2011). These conflicting result might be due to differences in the intensity of resistance training. Therefore, resistance training might need to be carefully prescribed based on individual pre-existing conditions and the anticipated outcome of the exercise program. Moderate and low intensity resistance training is recommended from the general viewpoints of health promotion and safety.

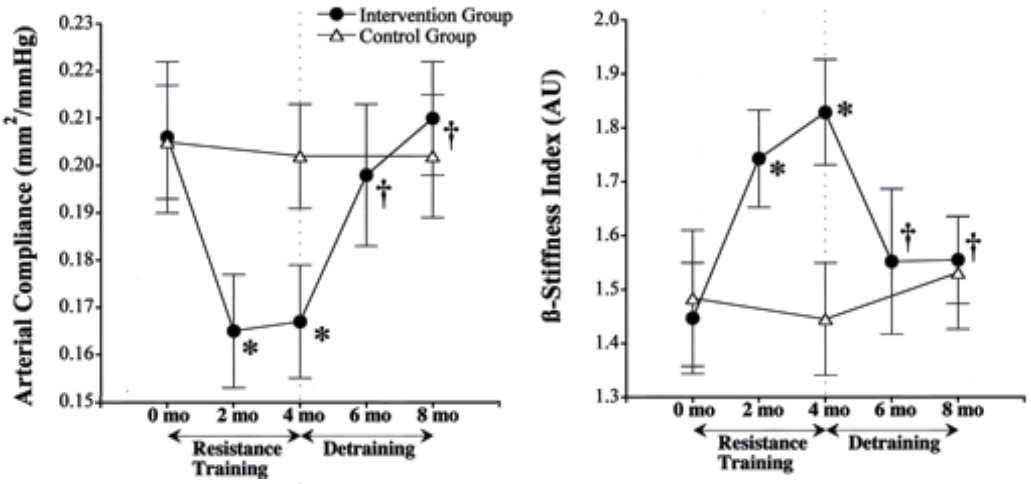

Fig. 7. Changes in carotid arterial compliance (top) and β-stiffness index (bottom) in the intervention group (black circles) and control group (white triangles). Values are mean±SEM. *P<0.05 vs baseline; †P<0.05 vs resistance training period (2- and 4-month values) (Quotation from Miyachi et al. 2004).

6.3 Other types of exercise and arterial stiffness

Physical activity stimulates increases in bone diameter throughout life and diminishes the risk of fractures by mechanically counteracting the rates of bone thinning and bone porosity. Exercise can be associated with an increase in muscle contraction and thus with more strain applied to bone, which is important for bone mass stimulation. Whole body vibration has been investigated from the viewpoints of sport, rehabilitation and treatment for osteoporosis. Whole-body vibration is a new training modality that increases muscle strength and mass to the same extent as resistance training at moderate intensity, which can be of clinical importance in individuals who cannot perform high-intensity and prolonged traditional exercise. Whole body vibration acutely decreases arterial stiffness (Otsuki et al. 2008, Fig. 8). Moreover, whole-body vibration prevents increases in leg arterial stiffness and attenuates increases in systemic arterial stiffness (Figueroa et al, 2011). Thus, whole body vibration is beneficial not only to the skeletal system and musculature but also to the cardiovascular system.

Whole body vibration is feasible not only in healthy humans but also in vulnerable populations such as those with osteogenesis imperfecta (Semler et al, 2007). Whole body vibration reflexes to the lumbar spine can be induced by upright standing on a vibrating platform. The application of vibrations increased bone formation and the metabolism in skeletal muscles and skin (Bleeker et al, 2005; Kerschau-Schindl et al, 2001). As whole body vibration -induced oscillation is propagated at least to the lumbar spine (Rubin et al, 2003), it is reasonable to consider that whole body vibration mechanically stimulates abdominal and leg arteries. Therefore, whole body vibration may reduce arterial tone and decrease arterial stiffness via mechanical stimuli to arteries.

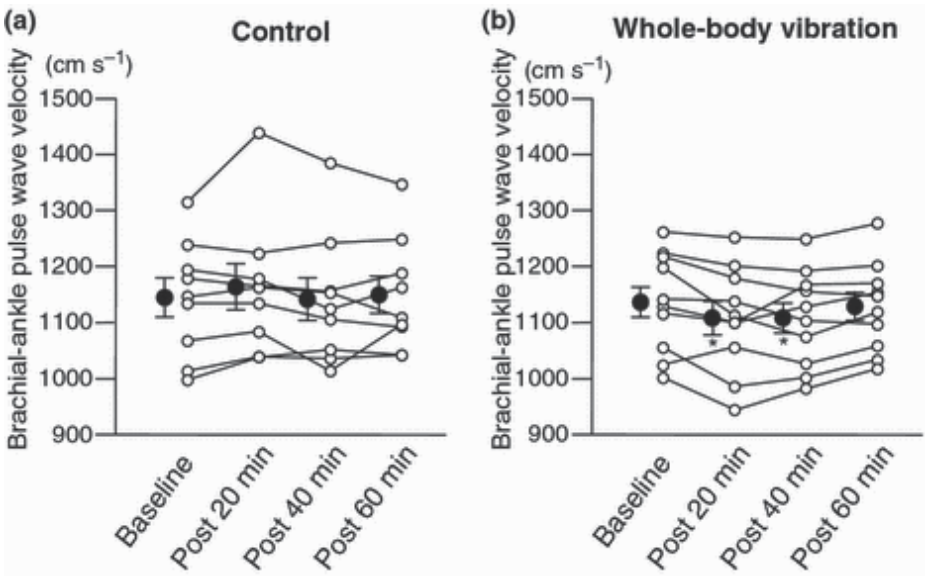

Fig. 8. Brachial-ankle pulse wave velocity (baPWV), an index of arterial stiffness, before and 20, 40 and 60 min after control (a) and whole-body vibration (WBV, b) sessions. Open circles are individual values and closed circles are mean ± SE. *$P < 0.05$ vs. baseline (Quotation from Otsuki et al. 2008).

7. Summary

Based on these results, we encourage the clinical prescription of specific exercise programs to impede the progression of osteoporosis and/or atherosclerosis and to confer health benefits that will assure a better long-term quality of life and decrease the public health burden.

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27

Osteoporotic Pain

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1. Introduction

Pain derived from musculoskeletal disorders play a major role in the health profile of the general population (Badley et al., 1994). Generally, osteoporosis patients experience several kinds of pain, including LBP: pain derived from external injury such as from fractures of the compressed vertebrae or femoral neck, and pain from internal consequences of the osteoporotic state without injury, which has been reported to account for pain in 89% of menopausal osteoporosis patients (Scharla et al., 2006). The exact mechanism for that pain still remains unknown, but some studies have tried to clarify that. In a previous study in which SPECT RI, bone scintigraphy, and X-rays were used, pain from injury was reported to be caused by collapsed vertebral bodies and degenerated intervertebral disc and facet joints (Ryan et al., 1992), which proved one of the evidence of injury-derived pain. The injury-derived pain in osteoporosis patients tends to turn into acute pain, whereas the non-injury-derived pain tends to take a chronic course, among which the latter must be sought for its pathogenesis. Here, we define pain derived from osteoporosis without any fractures or injuries as "osteoporotic pain." In this chapter, we review osteoporotic pain by showing the association between its possible mechanism and treatment. Regarding the detailed pharmacological character and use of each anti-osteoporosis agent, please refer to the other appropriate chapters.

2. Mechanism and pharmacological management of osteoporotic pain

2.1 Overview

In the bone tissue, nociceptors respond to mechanical, thermal, and chemical stimuli. Injury or inflammation results in the release of a variety of chemical mediators (e.g., prostaglandins, cytokines, and growth factors), which not only stimulate osteoclast activity but also activate nociceptors and decrease their threshold for activation (Haegerstam, 2001; Payne, 1997). The alteration in bone turnover leads to microfractures of bone, which may be one of the possible accepted origins of osteoporotic pain. Furthermore, other mechanisms for osteoporotic pain are reviewed in this section.

Menopause is well known to be one of the essential causes of osteoporosis in humans (Albright, 1989), and the most important change after menopause is the depletion of estrogen that regulates the expression of various genes (Beato, 1989), which leads to a decrease in the amounts of gene products, including receptors and peptides, required for

modulation of nociceptive transmission. Furthermore, estrogen modulates osteoclast formation both by directly suppressing Receptor activator of NF-κB ligand (RANKL)-induced osteoclast differentiation and by down-regulating the expression of osteoclastogenic cytokines from supportive cells (Shevde, et al., 2000).

In basic studies, ovariectomized (OVX) rats are often used as well-known osteoporosis pathological model, which exhibit the same hormonal changes observed in humans with osteoporosis. Regarding pain perception, a significant reduction in the latencies of tail withdrawal from hot water (Forman et al., 1989) and long-term formalin-induced licking has also been reported to be increased in OVX rats (Franceschini et al., 1983), and because of this, OVX is thought to induce hyperalgesia in rats. These increased pain perception should be another reason for the osteoporotic pain.

Osteoporosis treatment against pain itself potentially includes the prevention of possible fracture-induced pain by increasing bone mass density (BMD), which each agent originally aims to acquire. Furthermore, each anti-osteoporosis agent has been reported to have its own specific pain-related active site, which will be described further in the following sections.

In this section we will review the possible mechanism underlying osteoporotic pain with the relation to the osteoporosis agents, details of which have been obtained from several studies in which some of the mechanisms overlap and remain unclear, that tells us the several sources of osteoporotic pain in the central/peripheral nervous system for its manifestation in local sites of osteoporosis. Fig. 1 below shows us the general view of several sources of osteoporotic pain in the central/peripheral nervous system. Regarding the detail of the each agent, please refer to the following subsections.

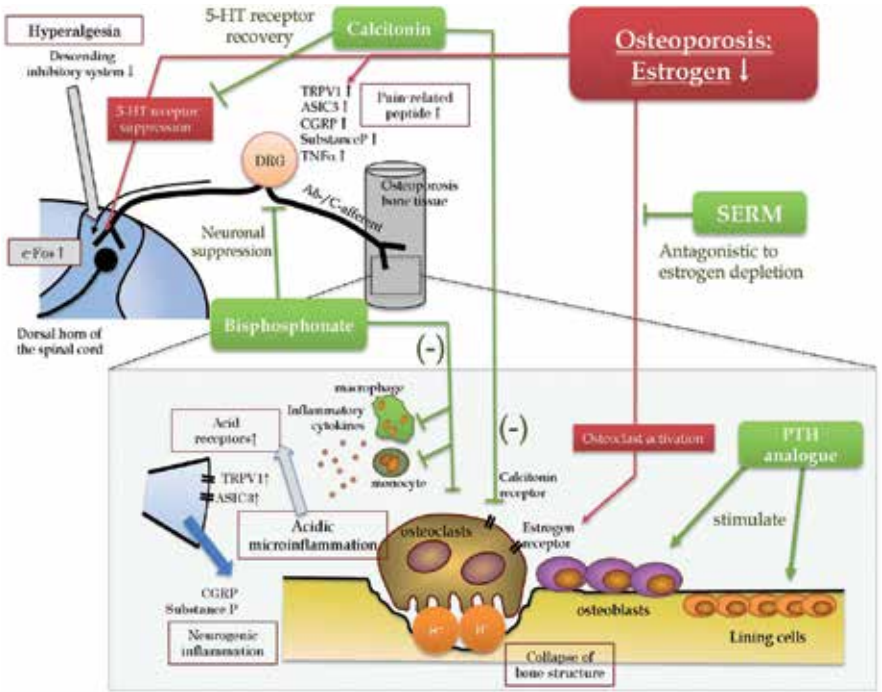

Fig. 1. Possible mechanism underlying osteoporotic pain. Green boxes show the roles of osteoporosis agents.

2.2 Calcitonin

Calcitonin is a polypeptide hormone involved primarily in the regulation of calcium homeostasis; it is secreted into the general circulation by the parafollicular C cells of the mammalian thyroid gland, and regulates the blood calcium concentration and bone metabolism by suppressing the activity of osteoclasts by binding calcitonin receptor on them. Thus, it reduces the blood calcium concentration in hypercalcemia and improves bone mass in osteoporosis. It is usually administered via a subcutaneous injection, and its analgesic effect as well as the resulting increase in bone mineral density (BMD) has been observed and reported in clinical situations; some RCT studies showed that calcitonin produced an analgesic effect in patients with osteoporotic vertebral compression fractures (Knopp et al., 2005; Lyritis et al., 1999), reflex sympathetic dystrophy(or Complex regional pain syndrome: CRPS) (Gobelet et al., 1992) , and cancer pain (Roth & Kolarić, 1986).

The analgesic effect of calcitonin is reported to be related to the serotonergic system in the spinal cord: a presynaptic serotonin (5-HT)-induced inhibition of excitatory glutamatergic transmission evoked monosynaptically by stimulating C-afferent fibers in the substantia gelatinosa (SG) neurons existing in the lamina II of the spinal dorsal horn (Fig. 2). Incidentally SG neurons play an important role in the modulation of nociceptive transmission from the periphery to the central nervous system (CNS), in which nociceptive information is transmitted by fine myelinated Aδ-afferent and unmyelinated C-afferent fibers terminating preferentially (Kumazawa & Perl, 1978; Light et al., 1979; Sugiura et al., 1986; Sugiura et al., 1989; Yoshimura & Jessell, 1990; Yoshimura & Jessell, 1989).

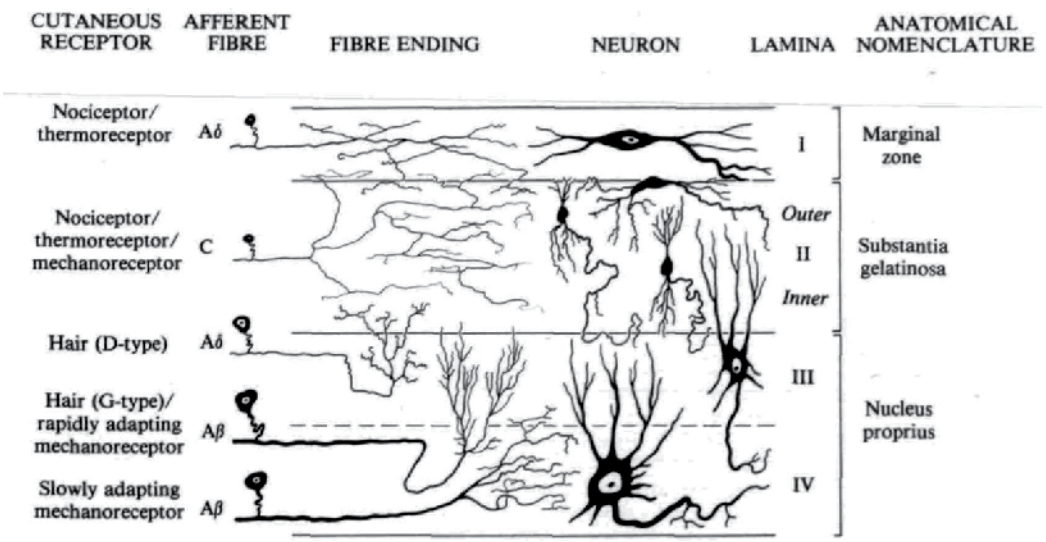

Fig. 2. Schematic diagram of the neuronal organization of the superficial dorsal horn in the spinal cord and the afferent input to the same. Substantia gelatinosa exists in lamina II, in which myelinated Aδ-afferent fibers and unmyelinated C-afferent fibers terminate preferentially (Cervero & Iggo, 1980)

In osteoporosis, estrogen deficiency not only causes bone loss but also alters the spinal serotonergic system by suppressing 5-HT receptor expression, which usually plays an important role in descending pain inhibitory system; this results in hyperalgesia. In other words, the hyperalgesia observed in the osteoporotic model is, at least in part, mediated by

disinhibition of pain transmission in the spinal cord. Calcitonin recovers these changes in the dorsal horn leading to a resumption of synthesis of 5-HT receptors followed by the recovery of descending inhibiting pathway; this in turn produces the analgesic effect (Ito et al., 2000).

Other previous studies demonstrate the analgesic effect of calcitonin. One basic study shows that calcitonin decreased hyperalgesia in ovariectomized rats by upregulating the activity of the descending serotonergic inhibitory system (Takayama et al., 2008) , and another clinical study reported that it produced an effect comparative to morphine analgesia (Martin et al., 1995) . Furthermore, calcitonin has been reported to significantly increase the plasma β-endorphin levels in patients with postmenopausal osteoporosis (Ofluoglu et al., Akyuz, Unay, & Kayhan, 2007) . These facts prove the analgesic effect of calcitonin in osteoporotic pain.

Additionally, calcitonin is administered subcutaneously in the clinical situation. That makes easier to use for those osteoporotic pain patients with symptoms of gastroesophageal reflux disease and in elderly patients with kyphosis (Yamane et al., 2011) or with low medical compliance, which often makes it difficult to use other internal agents.

2.3 Bisphosphonate

Bisphosphonate (BP) regulates bone turnover by suppressing osteoclast activity, and its anti-fracture efficacy has been reported in osteoporosis patients. BP exerts its anti-osteoporosis effects by binding to hydroxyapatite in the bone tissue, inhibiting osteoclast activity, and inducing apoptosis of osteoclasts. Recently, it has been reported to produce suppressive effects on monocytes and macrophages as well; this in turn leads to the suppression of more acute conditions (Roelofs et al., 2010)

Clinically BP has the potential to prevent or relieve back pain in patients with spinal osteoporosis. For instance, risedronate produced an analgesic effect on osteoporosis patients with chronic low back pain who had no evidence of fractures (Ohtori et al., 2010). Alendronate resulted in a rapid decrease in back pain and improvement in QOL in postmenopausal women with osteoporosis (Iwamoto et al., 2010). In addition, an RCT study showed that alendronate produced a stronger analgesic effect than calcitonin in postmenopausal osteoporotic women (J. Iwamoto et al., 2010).

Recent studies tell us that several factors are involved in the analgesic mechanism of BP. First, it is caused by the modulation of pain-transmitting neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) and inflammatory cytokines such as tumor necrosis factor (TNF)-α. Regarding the effect on pain-related neuropeptide, ibandronate is reported to suppress the expression of substance P mRNA and TNF-α in dorsal root ganglia (DRG) in a rat model of persistent inflammation (Bianchi et al., 2008). Here estrogen has reported to suppress CGRP production in DRG using OVX rats (Yang et al., 1998); hence, it is acceptable that estrogen deficiency in osteoporosis patients induces increased CGRP production. Herein we demonstrated that risedronate has suppressed the CGRP production (Fig. 3).(Orita et al., 2010).

Also, transient-receptor potential vanilloid 1 (TRPV1) is also upregulated in the DRGs of OVX rats (Orita et al., 2010). TRPV1 is a ligand-gated non-selective cation channel preferentially expressed in small-diameter primary afferent neurons (Tominaga et al., 1998). It responds to capsaicin, noxious heat and acid. Osteoporotic osteoclasts degrade bone minerals by secreting protons through the vacuolar H^+-ATPase creating local acidic

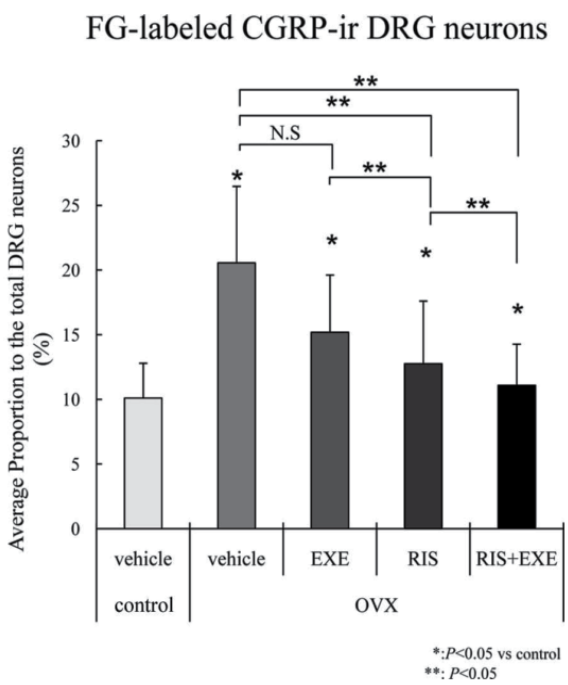

Fig. 3. CGRP production in ovariectomized (OVX) rats. Average CGRP production is suppressed in the BP-treated group than in the vehicle-treated OVX group or physical exercise (EXE)-only treated group. TheCGRP production was mostly suppressed by the combination of RIS and EXE (described in section 3) (Orita et al., 2010).

microenvironments by inflammation (Rousselle & Heymann, 2002; Teitelbaum, 2000), which should evoke the stimulation of TRPV1. Furthermore, this acidic microenvironment stimulates acid-sensing ion channels (ASICs). Increased activities of osteoporotic osteoclasts also lead to these upregulation of pain-related nociceptors and channels; hence, BP should downregulate their activity by suppressing osteoclasts. Indeed, the effect of BP on the increased number of TRPV1 has not been clarified; however, BP should have an effect on TRPV1 because the receptor has been reported to modulate the synthesis and release of CGRP in sensory nerves. Furthermore, activation of these pain-related molecules induces increased production of c-Fos protein in the spinal dorsal horn, which is expressed by both noxious and non-noxious stimuli in the postsynaptic neurons of the spinal dorsal horn (Hunt et al., 1987). It is upregulated in response to various stimuli from the primary afferent neurons (Hunt et al., 1987; Menétrey et al., 1989; Morgan & Curran, 1991), thus it is used for a marker for neuronal excitation including pain. These findings such as increased production of pain-related channels, receptors, and proteins in DRG and activated spinal cord should be the another reason for osteoporotic pain.

Furthermore, BP is indicated to have a direct suppressive effect on pain-related sensory neurons. We demonstrated that risedronate inhibited axonal growth of neurite of pain-related small-sized DRG neurons isolated from rat neonates *in vitro* (Orita et al., 2010). The underlying mechanism remains unclear, but BP itself can produce an analgesic effect in osteoporotic patients by suppressing peripheral nerve function.

The analgesic effect of BP has come to be studied and recognized as reviewed here. Hence, BP can be a useful agent for dealing with osteoporotic pain.
When using BP, we have to be careful of its side effects such as gastroesophageal reflux disease in elderly patients with kyphosis, and jaw necrosis. However, BP should be of use after the exclusion of these possible side effects.

2.4 Hormone replacement treatment (HRT) and selective estrogen receptor modifier (SERM)

Estrogen deficiency is the most major pathology in osteoporosis. Thus there should be suggesting that hormone replacement treatment (HRT) could be an alternative treatment. However HRT is not recommended by several studies for its side effects: breast cancer, coronary heart disease, stroke, and pulmonary embolism (Rossouw et al., 2002). Regarding pain, several clinical reviews indicate that the low back pain treatment with HRT is not significantly effective (Symmons, et al., 1991) and not recommended (Gamble, 1995; South-Paul, 2001; Willhite, 1998).
Instead of HRT using estrogen, selective estrogen receptor modifier (SERM) has been used for the treatment and prevention of osteoporosis. Raloxifene is a benzothiophene-derivative SERM that binds to estrogen receptors α and β and exerts estrogen agonist effects or antagonist effects, depending on the target tissue: in bone tissue, raloxifene produces estrogen-like effects while it does not induce breast cancer(Cummings et al., 1999). Estrogen produces a suppressive effect on osteoclast activity by suppressing osteoclast differentiation and bone resorption (Luo et al., 2011). Hence, as an anti-osteoporosis agent, SERM increases BMD at the lumbar spine and hip region (Delmas et al., 1997), decreases bone turnover (Draper et al., 1996), reduces the risk of vertebral fractures in postmenopausal women with osteoporosis (Ettinger et al., 1999), and improves the lipid profile in healthy postmenopausal women (Walsh et al., 1998). Furthermore some possible mechanisms regarding the analgesic effect of raloxifene have been reported. First, it subserves the decreasing estrogen, which affects the sensitivity of nociceptive receptors (Hapidou & De Catanzaro, 1988) by facilitating pain production through pain-related neurotransmitters (Duval et al., 1996; Kawata et al., 1994). Second, pain modulation via central interactions using the endogenous opioids pathway system is reported (Quiñones-Jenab et al., 1997). By mimicking estrogen, raloxifene increases the number of glutamate receptors in the rostral cortex, nucleus accumbens, and striatum (Cyr et al., 2001), which are regions of the brain that have recently known to be involved in the nociceptive processing system (Chudler & Dong, 1995). Also, raloxifene affects dopamine receptors in the striatum and nucleus accumbens (Landry et al., 2002), which play an important role in nociception in acute and chronic pain conditions (Magnusson & Fisher, 2000). Furthermore, clinical studies suggested that raloxifene produces estrogen-like upregulating effects on plasma levels of β-endorphin (Florio et al., 2001), which acts as a neurotransmitter in the endogenous antinociceptive system. Hence, raloxifene affects nociceptive processing in CNS, possibly producing an analgesic effect. In addition, osteoclasts suppressed because of the estrogen-like effect of raloxifene should produce an analgesic condition through the reduced secretion of cytokines and reduce the risk of fractures.
While one study reported that raloxifene produced an analgesic effect in osteoporosis patients (Fujita et al., 2010), another study reported that the effect produced was not significant (Papadokostakis et al., 2006). This instability in estrogen or its alternative treatment should be due to the gradual decrease of estrogen receptor after menopause. And this shows that SERM should have some analgesic effect but might be better to be used in

combination with other osteoporosis treatment strategies to alleviate pain. Recently a new SERM, bazedoxifene, has been used in the clinical situation. Its analgesic effect is also should be investigated for osteoporotic pain patients.

2.5 Parathyroid hormone (PTH) and PTH analogue

Parathyroid hormone (PTH) stimulates bone formation by increasing the number of osteoblasts, partly by delaying osteoblast apoptosis (Jilka, 2007). Teriparatide, a recombinant of human PTH fragment 1-34 [rhPTH(1-34)], act as a bone anabolic agent which prevents, arrests, or partially reverses bone loss inducing new bone formation and improving bone microarchitecture (Peiqi Chen et al., 2007; Dempster, et al., 1993; Neer et al., 2001). The detailed mechanism of action of rhPTH is still under investigation, however the drug probably affects multiple pathways and alters the activity of osteoblasts, bone lining cells and osteocytes. Bone formation induced by PTH analogues not only increases BMD or bone mass but also improves the microarchitecture of the skeleton, thereby leading to improved bone strength and mechanical resistance (Kraenzlin & C. Meier, 2011). Hence, teriparatide has come to be used as one of the few anabolic agents for osteoporosis. A previous study reported that osteoporosis patients treated with teriparatide showed a greater analgesic effect on LBP than alendronate (Miller et al., 2005). Recently, a meta-analysis of five teriparatide trials showed that patients randomized to teriparatide had a reduced risk of new or worsening back pain during the active treatment phase compared with patients randomized to placebo or antiresorptive therapy (Nevitt et al., 2006).

Teriparatide can increase or decrease bone mass, depending on the mode of administration (Hock & Gera, 1992; Podbesek et al., 1983). Continuous infusions, which result in a persistent elevation of the serum parathyroid hormone concentration, lead to greater bone resorption than do daily injections, which cause only transient increases in the serum parathyroid hormone concentration(Tam et al., 1982). A previous study reported that a dose of 40 μg increased BMD to a greater extent than a dose of 20 μg but had similar effects on the risk of fracture and was more likely to produce side effects such as nausea and headache (Neer et al., 2001); this shows that physicians should be careful in prescribing the agent.

2.6 Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are commonly used in clinical situations to reduce inflammation and pain. The mechanism of NSAIDs is mainly based on inhibition of cyclooxygenase (COX) enzymes, which convert arachidonic acid into prostaglandins (PG). In particular the COX-2 isoform is accepted as a proinflammatory enzyme that is induced by inflammatory stimuli and responsible for the generation of proinflammatory PGE_2 (Niederberger et al., 2008). PGE_2 induces proliferation and activation of osteoclasts via osteoblast activation, hence its inhibition can lead to inhibiting osteoclast formation, which lead to analgesic effect (Kaji et al., 1996). However, they are often ineffective on osteoporotic pain because osteoporotic pain involves multiple mechanisms described above. Hence, clinical physicians dealing with pain should consider the existence of osteoporotic pain when NSAIDs are barely able to produce an analgesic effect on patients complaining of chronic pain for several months. Such patients would be an osteoporosis patients with osteoporotic pain who have no evidence of injuries (Ohtori et al., 2010). Long-term administration of NSAIDs can produce some side effects such as gastric ulcers or renal function disorder; hence, physicians should be careful when prescribing NSAIDs to pain patients and should always try to target the origin of their pain.

3. Non-pharmacological treatment and osteoporotic pain

Non-pharmacological treatment strategies such as physical exercise, nutrition, diet, and following of certain habits are also used. These non-pharmacological approaches can improve BMD by preventing a fracture. Considerable evidence indicates that physical exercise can be most useful among these approaches. The major objective of physical exercise in the prevention or treatment of osteoporosis is to reduce the incidence of fractures. Additionally it has been reported that physical exercises produce an analgesic effect for osteoporotic pain besides bringing about improved physical function and vitality (Li et al., 2009). A basic study using OVX rats showed that physical exercise (5 days a week for 30 min on a treadmill for 30 days) led to a significant decrease in CGRP production when combined with risedronate; this combination suppressed CGRP production more than risedronate alone.Furthermore, this combination led to the maximum improvement in BMD (Fig. 3) (Orita et al., 2010). This is attributable to the activation of osteoblasts by both BP and physical exercise. BP is reported to increase total cellular protein, alkaline phosphatase activity, and type I collagen secretion in vitro (Iwamoto et al., 2005), and adequate mechanical stress is reported to activate osteoblasts (Ban et al., 2011); this is the reason why BP and exercise make an effective combination, which coincides with that of a previous report (Fuchs et al., 2007; Tamaki et al., Akamine et al., 1998). Other combinations of physical exercise and osteoporosis treatment strategies should be effective. However, another study reports that excessive physical exercise such as running for long periods has a negative effect on bone metabolism and proinflammatory status, and leads to increased osteoclast activity and elevated production of TNF-α and interferon-γ by CD8+ T cells (Sipos et al., 2008); further, excessive physical exercise can lead to fractures. Hence, the medical staff should suggest physical exercise programs suited to each patient.

4. Conclusion

Osteoporotic pain is a clinically-known condition, but investigation of its mechanism and origin has only been performed in recent years. Osteoporosis treatment predominantly aims to increase the BMD of patients in order to prevent possible fragile fractures that sometimes lead to a critical condition or result in a poor quality of life (QOL). Considerable evidence shows that using pharmacological or non-pharmacological treatment strategies for these patients not only improve their BMD but also relieve their pain. Physicians should always bear these matters in mind when choosing a treatment strategy that would best benefit patients with osteoporotic pain.

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28

Pharmacological Treatment of Osteoporosis

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1. Introduction

Osteoporosis can be classified into two big categories, clinical osteoporosis and densitometric osteoporosis. Clinical osteoporosis involves a fragility fracture, and no densitometry is needed to start treatment. Densitometric osteoporosis is defined by means of a bone mineral density assessment. Treatment should be considered depending on the global fracture risk, and taking the densitometric results into account.
The first step in the pharmacological treatment of osteoporosis is to identify whether it is a primary disease or whether the bone mass loss is secondary to another disease. In the case of a secondary osteoporosis, treatment of the primary disease is the most important step. Pharmacological treatment should then be considered if the fracture risk is too high. The purpose of pharmacological treatment in osteoporosis is to reduce the risk of fracture. According to the World Health Organization's more than half of the patients suffering a fragility fracture do not have densitometric osteoporosis. (Siris et al., 2004) When any medication is started for osteoporosis treatment it must be remembered that this illness will require treatment for a long time and that the drug has to be given in conjunction with advise regarding lifestyle changes. It is therefore imperative to evaluate and make decisions based on issues such as cost, evaluation of cost-efficiency, and patient adaptability to drug safety.

2. Antiresorptives

2.1 Calcitonin

Calcitonin is a 32 amino acid polypeptide. It binds to osteoclasts and inhibits bone resorption. Calcitonins from many species are effective in humans, but salmon calcitonin is the most widely used. It is extremely potent in humans due to its higher affinity (forty times that of human calcitonin) for the human calcitonin receptor. The only other calcitonin clinically used is human calcitonin, less potent but also less antigenic than salmon calcitonin. (Carstens & Feinblatt, 1991) It can be administered by intramuscular, intravenous or nasal route. The bioavailability of nasal salmon calcitonin is only about 25 percent that of intramuscular calcitonin; thus, the biological effect of 50 international units (IU) of intramuscular salmon calcitonin is comparable to that of 200 IU of nasal salmon calcitonin. (Overgaard et al., 1991)

2.1.1 Clinical data

There is evidence that calcitonin is effective in the treatment of established osteoporosis. In one study of calcitonin in osteoporosis, 208 elderly osteopenic women were treated with calcium and either intranasal placebo, or 50, 100, or 200 IU of daily salmon calcitonin for two years. Mean spine bone mineral density (BMD) was increased by salmon calcitonin in a dose-dependent manner, and a maximum effect was seen with the 200 IU dose. (Overgaard et al., 1992)

The largest clinical trial with calcitonin for the treatment of osteoporosis was a five-year trial comprising 1,255 women with a lumbar spine T score of <-2 and at least one vertebral fracture. They were randomly assigned to placebo or 100, 200, or 400 IU of intranasal calcitonin per day. There was a small increase in spine BMD (1% to 1.5%) in all groups. The risk of vertebral fracture was significantly lower than placebo only in the group taking 200 IU per day, and the risk of non-vertebral fractures was significantly lower than placebo only in the group taking 100 IU per day. Thus, the beneficial effect of nasal calcitonin on vertebral BMD and vertebral fracture risk was small and inconsistent. (Chesnut et al., 2000)

Nowadays calcitonin is not a current therapy for osteoporosis. It has been displaced by other treatments. However, one beneficial short-term effect of calcitonin therapy is pain reduction in patients who have sustained a fracture. In one study, looking for pain effect of calcitonin, 56 osteoporotic women who sustained an atraumatic vertebral fracture were randomly assigned to treatment with placebo or 100 IU of intramuscular salmon calcitonin daily for two weeks. Mean pain scores and analgesic consumption in the calcitonin group were significantly lower than in the placebo group by the fourth day. Similar benefits on bone pain have been observed in several other small, randomized trials of parental and nasal calcitonin. The ability to relieve pain may represent a truly distinguishing feature from other drugs used in the treatment of osteoporosis and maybe today it is one of its main indications. (Lyritis et al., 1991)

2.1.2 Adverse effects

The most frequent adverse effects of calcitonin appear during or shortly after its parenteral administration: digestive disorders, nausea, vomiting, abdominal pains, diarrhea, vasomotor disorders or facial flushing among others. Allergy to calcitonin is possible but exceptional. Thus, calcitonin may be the antiresorptive agent of choice in patients with pain from an acute osteoporotic fracture. Why pain relief occurs is not well understood; one possibility is a rise in endorphin levels induced by calcitonin.

2.2 Hormonal replacement therapy (HRT)

The HRT is a treatment option that includes different estrogen doses, in combination, or not, with progestagens. Hormonal replacement therapy is described in detail in another chapter. Therefore, in this chapter we are just going to make a review of the antifracture efficacy of HRT along with the safety data from different meta-analysis, systematic reviews and clinical guides.

2.2.1 Hormone replacement therapy (HRT) for fracture prevention

The estimations of fracture risk, derived from the principal cohort studies of postmenopausal women, using HRT for long periods of time, show a significant vertebral fracture risk reduction (RR=0.6; CI 95%: 0.36 to 0.99) and wrist fracture risk reduction

(RR=0.39; CI 95%: 0.24 to 0.64), but a non-significant hip fracture risk reduction (RR=0.64; CI 95%: 0.32 to 1.04). The WHI study (Women's Health Initiative), a randomized clinical trial (RCT) that evaluated postmenopausal women randomized to combined HRT (combined equine estrogen 0.625 mg daily plus medroxyprogesterone 2.5 mg daily) or placebo, demonstrated, after 5.2 years of treatment, a hip fracture risk reduction of 34% (hazard ratio [HR]=0.66; CI 95%: 0.45 to 0.98), of clinical vertebral fractures of 34% (HR=0.66; CI 95%: 0.44 to 0.98) and a reduction in any fracture of 24% (HR=0.76; CI 95%: 0.69 to 0.85). (Rossouw et al., 2002; Cauley et al., 2003) In the same study, the branch using estrogen alone showed similar results, but it was suspended due to an unfavorable risk benefit ratio. (Anderson et al., 2004) In two meta-analysis of RCTs a reduction of 27% (RR=0.73; CI 95%: 0.56 to 0.94) in non-vertebral fractures and a tendency towards the decrease of vertebral fractures (RR=0.66; CI 95%: 0.41 to 1.07) was described. (MacLean et al., 2008) Nevertheless, from the HERS (The Heart and Estrogen + Progestin Replacement Study) RCT and from the cohort followed, the HERS II study (Hulley et al., 1998), no reduction of the risk of hip fractures or of other locations (RR=1.04; CI 95%: 0.87 to 1.25) in women with cardiovascular disease history, could be demonstrated. (Cauley et al., 2001)

Another meta-analysis, which included information from two publications of the WHI study (women with and without osteoporosis, without DMO measurements) found a reduction of 25% of the relative risk of non-vertebral fractures (RR=0.75; CI 95%: 0.70 to 0.81) in a sample of 31,333 patients followed up for a maximum of 7 years and a 36% relative risk reduction for hip fractures (RR=0.64; CI 95%: 0.49 to 0.84) in a sample of 27,347 patients. (Liberman et al., 2006) The British National Institute of Health and Clinical Excellence presented a meta-analysis of RCTs on the efficacy of HRT (with estrogen alone or combined) vs. placebo / not treatment in postmenopausal women or with surgical menopause. (National Institute for Clinical Excellence [NICE], 2008) The results were presented by fracture location and specified the RCT used for the estimation of the relative risk. The results are summarized in table 1.

2.2.2 Safety

2.2.2.1 Vascular illness

A systematic review of five RCTs, studying HRT with estrogen and two with combined HRT estrogen plus progesterone, did not demonstrate significant differences in the incidence of acute coronary events (including acute myocardial infarction) between the group of intervention and the control group. (MacLean et al., 2008) Two of the essays with estrogen and two of combined therapy (estrogen plus progesterone) that reported the incidence of death of cardiac origin did not demonstrate significant differences between the intervention group and control group. Combined results of three essays comparing estrogenic therapy with placebo (Anderson et al., 2004; Mosekilde et al., 2000; Cherry et al., 2002) presented an odds ratio (OR) of 1.34 (IC 95%: 1.07 to 1.68) for cerebral vascular events. The combined results of the essays that compared combined therapy estrogen plus progesterone with placebo (Rossouw et al., 2002; Hulley et al., 1998), demonstrated a higher risk of ictus (OR=1.28; CI 95%: 1.05 to 1.57) in the intervention group. Of 4 systematic reviews of observational studies in women receiving HRT (Stampfer et al., 1991; Grady et al., 2002; Barrett-Connor, 1992; Humphrey et al., 2002), three demonstrated an important reduction in the global risk of mortality for acute coronary events. The most recent systematic review, that controlled selection bias of inclusion and analysis, did not show any

association between the THS and the incidence, and mortality of acute coronary events. (Humphrey et al., 2002)

The WHI primary prevention study showed a significant increase in the risk of acute coronary events (41%), beginning the second year of treatment (29 cases in the treatment group, compared with 21 cases for 10.000 women/year in the general population). (Rossouw et al., 2002) This increase was higher in non-mortal coronary events (RR=1.50; CI 95%: 1.08 to 2.08) than in the mortal coronary events (RR=1.20; CI 95%: 0.58 to 2.50). The RCTs of HRT with estrogens alone, in primary prevention as well as in secondary prevention, did not show any benefit on the cerebrovascular illness. (Viscoli et al., 2001) The group of the WHI study with estrogen also showed an increase in the risk of cerebrovascular accidents. (Anderson et al., 2004)

Fracture Location	Nr of RCTs	n	RESULTS	References
Vertebral fracture	4 RCTs	11,842	RR=0.55; CI 95%: 0.46 to 0.66	(Wimalawansa, 1998; Mosekilde et al., 2000; Anderson et al., 1997; Lufkin et al., 1992)
Non-vertebral fracture	3 RCTs	11,774	RR=0.73; CI 95%: 0.65 to 0.81	(Wimalawansa, 1998; Anderson et al., 2003; Mosekilde et al., 2000)
Hip fracture	2 RCTs	11,745	RR=0.63; CI 95%: 0.42 to 0.93	(Anderson et al., 2003; Mosekilde et al., 2000)
Any type of fracture	3 RCTs	11,556	RR=0.70; CI 95%: 0.63 to 0.78	(Anderson et al., 2003; Herrington et al., 2000; Ravn et al., 1999)

Table 1. Relative risk of fractures in meta-analysis from the NICE

2.2.2.2 Venous thrombotic events

In a systematic review, McLean et al., reported that estrogen treated patients present a higher risk of major venous thromboembolic events (OR=1.36; CI 95%: 1.01 to 1.86) compared to the placebo group. (MacLean et al., 2008) Another systematic review evaluating the effect of the HRT (estrogen with or without progestagens) included 12 studies (3 RCTs, 8 case-control studies and 1 cohort study) and showed an increase in the risk of thromboembolism (RR=2.14; CI 95%: 1.64 to 2.81). This risk is higher in the first two years of treatment and it is dose dependant. (Miller et al., 2002) The HERS study for secondary prevention showed an increase in the risk of thromboembolism in women with cardiovascular illness. (Hulley et al., 1998; Grady et al., 2002)

2.2.2.3 Breast cancer

A systematic review of 4 RCTs demonstrated that patients treated with estrogens alone present lower risk of breast cancer (OR=0.79; CI 95%: 0.66 to 0.93) compared to placebo. (MacLean et al., 2008) On the contrary, patients treated with estrogen and progestin present a higher risk of breast cancer (OR=1.28; CI 95%: 1.03 to 1.60) compared to placebo. (Rossouw et al., 2002; Hulley et al., 1998; Lufkin et al., 1992)

The combined HRT group of the WHI study, showed an increase in the risk of invasive breast cancer. (Rossouw et al., 2002) This increase took place after the fourth year of treatment (RR=1.26; CI 95 %: 1.0 to 1.59), with a tendency to increase according to the duration of the treatment (38 cases compared with 30 for 10,000 women/year). The patients in the treatment group were diagnosed in more advanced stages. No significant differences were found for the in situ carcinoma. (Chlebowski et al., 2003) Moreover, other studies have demonstrated that both the sequential and the continuous administration of the progestagens, collaborate to increase breast cancer. (Li et al., 2003)

2.2.2.4 Endometrial cancer

The administration of isolated estrogen increases the risk of developing endometrial hyperplasia and cancer. (Lethaby et al., 2004; Nelson, 2004) A meta-analysis including 29 observational studies showed a significant increase in the endometrial cancer risk with or without combined estrogens (RR=2.3; CI 95%: 2.1 to 2.5). (Grady et al., 1995) This risk is proportional to the duration of the treatment and remains elevated for up to 5 years or more after stopping treatment. In addition, an increase in endometrial cancer mortality was observed, but it was a non-significant increase (RR=2.7; CI 95 %: 0.9 to 8.0). The combined HRT group of the WHI study and its continuation for secondary prevention (HERS II) showed a relative risk of endometrial cancer of 1.58 without reaching statistical significance (IC 95%: 0.77 to 3.24). (Menopause and post menopause workgroup, 2004)

2.2.2.5 Ovarian cancer

In a systematic review of several case-control studies no association could be found between HRT and ovarian cancer. (Coughlin et al., 2000) In contrast, more recent systematic reviews of observational studies confirm the increase in the risk of ovarian cancer in women receiving treatment, especially long-term treatment (more than 10 years). (Garg et al., 1998; Negri et al., 1999) Two cohort studies of postmenopausal women treated for more than 10 years confirm this risk increase (RR=2.2; CI 95%: 1.53 to 3.17), as well as the mortality risk (RR=1.59; CI 95%: 1.13 to 2.25). (Lacey et al., 2002; Rodriguez et al., 2001) The combined HRT group of the WHI study also showed a non-significant increase in the risk of ovarian cancer (RR=1.58; CI 95 %: 0.77 to 3.24). (Anderson et al., 2003)

In summary, HRT is effective for the treatment of postmenopausal osteoporosis and the reduction of fracture risk. In spite of this, it is not advisable to use HRT (combined estrogen and progestagens) for more than 5 years due to the potential risks associated with the treatment of an equivalent dose of 50 picograms of estradiol per day. When HRT is indicated, it has to be prescribed at low doses (equivalent to estrogen transdermal patches of 25 mcg) and only if strictly necessary, higher doses. Estrogens and progestagens are recommended only in women with intact uteri. The progestagen dose must be calculated according to the estrogen dose. In those cases where a hysterectomy was performed due to endometrial cancer, HRT must not combine estrogen and progestagens. Continuous combined HRT must not be started until after one year of menopause.

2.3 Selective estrogen receptor modulators (SERMs)

So far two estrogen receptors have been described, alpha (ERα) and beta (ERβ), which are at different levels and locations in different body tissues. The ERβ are mainly in the developing spongy bone, while ERα are concentrated more on the cortical bone. In addition, these receptors have differences in their structure and function, which would explain other effects of estrogen deficiency as vasomotor symptoms or alterations in the lipid profile.

The selective estrogen receptor modulators are drugs with selective effects on the estrogen receptor. They can act as estrogen receptor agonists in some tissues while acting as estrogen receptor antagonists in others. SERMs embrace diverse molecules that lack the steroid structure of estrogens, but own a tertiary structure that allows them to bind to ERα and/or ERβ with different potency. In contrast to estrogens and estrogen receptor agonists, these are partial agonists/antagonists. Due to their selective estrogen-agonist properties on different tissues, SERMs may be indicated for the prevention or treatment of diseases caused by estrogen deficiency, like osteoporosis, without some of the adverse effects of estrogens. In addition, due to their selective properties in the breast (estrogen receptor antagonists), SERMs can be also utilized to prevent or treat breast cancer, where estrogen-agonistic activity is not wanted.

2.3.1 Differences between SERMs

Currently there are two types of SERMs that are differentiated by their chemical structure: triphenylethylene derivatives, such as tamoxifen and toremifene, and a benzothiophene derivative, raloxifene. The first two are used for the treatment of breast cancer while raloxifene is indicated for the prevention and treatment of osteoporosis. All have been associated with an increased incidence of pulmonary thromboembolism and with the onset of hot flushes but they have a beneficial effect on the lipid profile.

Tamoxifen is not indicated for the treatment of osteoporosis due to increased incidence of endometrial cancer associated with prolonged treatment and the weak effect of this drug on bone that is not maintained over time. The results of the studies that evaluated the effect of tamoxifen on fracture risk were contradictory.

The SERMs differ significantly in terms of tissue specificity. Bazedoxifene seems to have less effect on the uterus than estradiol and raloxifene in animal experiments due to lower estrogen receptor alpha agonistic effects. Tamoxifen and toremifene are used to treat breast cancer. Raloxifene is indicated for the treatment and prevention of osteoporosis and for the prevention of breast cancer. Besides the SERMs described in this review, other new SERMS have had clinical trials suspended prematurely: levormeloxifene, for causing urinary incontinence and uterine prolapse, arzoxifene for lacking effectiveness, and idoxifene, for resulting in increased endometrial thickness on ultrasonography but without significant histological abnormality.

2.4 Raloxifene

Raloxifene has estrogenic activity in bone and other systems but not in reproductive tissue. In ovariectomized animals, raloxifene preserves bone density, lowers serum total cholesterol, and inhibits aortic cholesterol accumulation, without causing endometrial hyperplasia. The mechanism of selectivity of raloxifene is not fully understood. There are studies that suggest that raloxifene has different effects than estradiol at the estrogen receptor. It also seems to have a different modulation in DNA response.

Several studies have demonstrated the effectiveness of raloxifene in the preservation of bone in early postmenopause. In a meta-analysis of seven trials (four treatment and three prevention trials) examining the effects of raloxifene versus placebo on bone mineral density, raloxifene increased bone mineral density of the lumbar spine after two years of treatment. (Cranney et al., 2002) A study with 601 women, five years after menopause, that received a daily treatment with 30, 60 or 150 mg of raloxifene for two years, showed an

increase in bone mineral density in spine and hip, while the placebo was associated with reduced bone mineral density at the same sites. (Delmas et al., 1997) Compared to placebo, the average change in BMD with 60 mg of raloxifene was 2.4% at the spine and 2.4% at the total hip ($p < 0.001$ vs placebo). However, in two placebo-controlled trials with 145 5-year postmenopausal women 60 years or younger, treatment with raloxifene for three years showed a minor effect on spine and hip BMD. (Johnston et al., 2000) The change in BMD of the spine at three years was -1.32% with placebo, 0.71% with 30 mg of raloxifene, 1.28% with 60 mg raloxifene and 1.2% with 150 mg of raloxifene. Similar changes in hip BMD were observed in the respective treatment groups. In another analysis two studies involving 328 women with a mean age of 55 years and five years after menopause, treatment for five years with 60 mg of raloxifene was associated with preservation of BMD and a reduced risk of osteoporosis compared with the placebo group. The treatment with raloxifene compared to placebo showed an average increase in BMD of 2.8% at the lumbar spine and 2.6% at the hip (in both $p < 0.001$). (Jolly et al., 2003)

Raloxifene has shown to be effective in reducing the risk of invasive breast cancer in older women. Postmenopausal women with low bone mass and osteoporosis were studied in a trial named Multiple Outcomes of Raloxifene Evaluation (MORE, n=7,705) and its complementary study, called the Continuing Outcomes Relevant to Evista (CORE, n=4,011). (Burshell et al., 2008) In this study, women had an average age of 65 years (group with low bone mass) and 68 years (group with osteoporosis) and were followed for eight years. Regarding fractures, raloxifene reduced the risk of vertebral fracture; however, it did not show a reduction in non-vertebral fractures. Moreover, in a meta-analysis of RCTs comparing raloxifene to placebo, raloxifene consistently reduced the risk of vertebral fractures in postmenopausal women (OR=0.6; CI 95%: 0.5-0.7).

In the MORE trial, a subset of 6,828 of the women had lumbar spine x-rays at baseline and after 36 months of treatment. Among the women receiving 60 mg and 120 mg raloxifene new vertebral fractures were observed in 6.6% and 5.4%, respectively, compared with 10.1% in the placebo group. The risk of non-vertebral fracture was similar in the three groups. After four years of raloxifene treatment (60 mg per day), the cumulative relative risk of one or more vertebral fractures was 0.64 (IC 95%: 0.53 - 0.76), compared with placebo.

Compared with placebo, treatment with 60 mg of raloxifene was associated with a reduction of 65 to 78% in the incidence of invasive breast cancer and breast invasive cancer with positive estrogen receptor (both $p < 0.05$). Therefore, the FDA approved raloxifene to reduce the risk of invasive breast cancer in postmenopausal women at high risk. (Barrett-Connor et al., 2006)

2.4.1 Combination therapy

The combination of alendronate and raloxifene resulted in a greater increase in BMD when compared with either drug alone. (Johnell et al., 2002) However, the benefit of combined versus monotherapy for fracture reduction is unknown and there are additional costs and side effects of taking two agents. As explained previously, some trials have reported that raloxifene (either taken concurrently or prior to PTH) does not suppress the BMD response to PTH as much as alendronate.

2.4.2 Adverse events

Several adverse events are associated with raloxifene. In the MORE and CORE studies an association between raloxifene and a 1.7 times increased risk of thromboembolism (TE),

compared with placebo, was observed (95% CI: 0.93-3.14; risk difference total of 0.9/1,000 women-years). (Martino et al., 2005) In a meta-analysis of nine studies, therapy with raloxifene was associated with an increase in the risk of deep venous thrombosis and pulmonary embolism (OR=1.5; CI 95%: 1.1-2.1 and OR=1.9; 95% CI: 1.0-3.5, respectively). (Adomaityte et al., 2008)

In the RUTH study (Raloxifene Use for The Heart), which included 10,101 postmenopausal women with coronary heart disease and an average age of 68 years, there was an association between Raloxifene and an increased risk of fatal stroke (HR=1.49; 95% CI: 1.00-2.24, an increase in the absolute risk of 0.7/1,000 women-years) and thromboembolism (HR=1.44; 95% CI: 1.06-1.95, an increase absolute risk of 1.2/1,000 women-years) compared with placebo. There was no increased risk of myocardial infarction or other coronary events in the RUTH study. However, as observed with thromboembolism and pulmonary embolism, the results of a recent analysis in a subgroup of the study showed an effect of age on incidence of coronary events, among women 60 years old or younger, the incidence of coronary events was significantly lower with raloxifene (50 cases), compared with the placebo group (84 cases; HR=0.59; 95% CI: 0.41 to 0.83, p=0.003). Raloxifene was also associated with an increase in hot flushes, particularly in women with new onset menopause. In MORE and CORE trials, 12.6% of women receiving raloxifene had hot flushes, compared with 6.9% in the placebo group ($p<0.0001$). (Collins et al., 2009)

In conclusion, raloxifene offers an alternative in the treatment of osteoporosis in selected patients. Its profile regarding heart disease and breast cancer is good but it should be carefully considered especially due to the high risk of venous thrombosis.

2.5 Bazedoxifene

Bazedoxifene is a novel, non-steroidal, indole based SERM that was developed using a rigorous preclinical screening process designed to select therapies with favorable effects on bone and lipid profiles while reducing the stimulation of uterus or breast tissue. (Komm et al. 2005; Komm & Lyttle, 2001) It is a third-generation SERM after the first generation tamoxifene, and the second-generation raloxifene. (Bazedoxifene: bazedoxifene acetate, 2008) Significant differences have been shown between the generations in terms of effects especially on the uterus and the breast tissue. (Vogel et al., 2006) It was developed using raloxifene as a template with the benzothiophene core substituted by an indole ring. (Gruber & Gruber 2004)

2.5.1 Pharmacokinetics and pharmacodynamics

Bazedoxifene is quickly absorbed with a t-max of approximately 2 hours and displays a linear increase in plasma concentrations after single doses from 0.5 mg up to 120 mg. (Chandrasekaran et al., 2009) It is highly bound (95.8% to 99.3%) to plasma proteins in vitro and it is extensively metabolized in women. Glucuronidation is the most important metabolic pathway. Slight or no cytochrome P450-mediated metabolism is apparent. Bazedoxifene-5-glucuronide is the major circulating metabolite and the concentrations of this glucuronide are approximately 10-fold higher than those of non-metabolized active substance in plasma. Bazedoxifene is excreted principally by feces and has a half-life of approximately 30 hours. Steady-state concentrations are achieved by the second week of once-daily administration. (Biskobing 2007; Shen et al., 2010) To study the bioavailability of bazedoxifene two oral formulations, a 10 mg tablet and two 5 mg capsules, and a 3 mg IV formulation were given to 18 postmenopausal women in a 3-way crossover design. Blood

was collected for 168 hours after each dose. The bioavailabilty of bazedoxifene was 6.2% for both oral formulations. (Patat et al., 2003) Finally, a study evaluated the longer-term pharmacokinetics of multiple doses of bazedoxifene. In a randomized, crossover study 23 postmenopausal women were given multiple doses of bazedoxifene (5, 20, 40 mg) for 14 days. Maximum concentration was achieved in 1–2 hours and t½ was approximately 28 hours. Protein binding was greater than 99%. Steady state concentrations were achieved by day 7. (Ermer et al., 2003)

2.5.2 Bazedoxifene in humans

Human studies with bazedoxifene have demonstrated a decreased thickness of the endometrium at doses of 30 to 40 mg/day compared to placebo or conjugated estrogen plus medroxyprogesterone (mean ± standard error of the mean increase after 168 days: 0.04 ± 0.12 mm for 30 mg, 0.12 ± 0.11 mm for 40 mg, 0.58 ± 0.21 mm for placebo, and 1.60 ± 0.23 mm for conjugated estrogen plus medroxyprogesterone acetate). (Ronkin et al., 2005) In a phase 2 study of healthy postmenopausal women, daily oral doses of bazedoxifene 2.5, 5.0, 10, 20, 30, or 40 mg were generally well tolerated and did not stimulate the endometrium. Furthermore, bazedoxifene 30 and 40 mg caused significantly smaller increases in endometrial thickness and significantly reduced the incidence of uterine bleeding compared with placebo. In a 2-year phase 3 study of postmenopausal women at risk of osteoporosis, bazedoxifene 10, 20, and 40 mg showed to prevent bone loss and reduce bone turnover and was associated with a favorable endometrial, ovarian, and breast safety profile. (Ronkin et al., 2005; Pinkerton et al., 2009; Miller et al., 2008; Archer et al., 2009)

A phase III, multicenter, double-blind, randomized, placebo-controlled study was designed exclusively to evaluate the efficacy of bazedoxifene for the prevention of fractures (Silverman et al., 2008). The study comprised 7,492 healthy postmenopausal women with osteoporosis with or without prevalent vertebral fractures. Participants were randomized to 20 or 40 mg per day of bazedoxifene, 60 mg of raloxifene, or placebo plus 1200 mg of calcium and 400 IU of vitamin D. The primary endpoint was the incidence of new vertebral fractures after 36 months. Secondary outcomes included, clinical vertebral fractures, worsening of vertebral fractures, non-vertebral fractures, breast cancer incidence, and changes in height. Both bazedoxifene 20 and 40 mg prevented the incidence of vertebral fractures with a similar efficacy as raloxifene when compared to placebo. The 3-year incidence of new vertebral fractures were 2.3%, 2.5%, 2.3%, and 4.1% in the bazedoxifene 20 mg, bazedoxifene 40 mg, raloxifene 60 mg, and placebo groups, respectively, with a significant reduction in relative risk for new vertebral fracture of 42%, 37%, and 42%, respectively, versus placebo. There was in general no effect on non-vertebral fractures, with incidence rates of 5.7% and 5.6% for the bazedoxifene 20 and 40 mg groups, respectively, compared with 5.9% for the raloxifene treatment group and 6.3% for the placebo group. Though, in a post-hoc analysis of women with higher risk for fractures (low femoral neck T-score and multiple vertebral fractures, n=1,772) bazedoxifene 20 mg demonstrated a 50% and 44% reduction in non-vertebral fracture risk compared with placebo (HR=0.50; 95% CI: 0.28–0.90; p=0.02) or raloxifene 60 mg (HR=0.56; 95% CI: 0.31–1.01; p=0.05), respectively. (Silverman et al., 2008)

2.5.3 Bazedoxifene and conjugated estrogen combination therapy

The rationale for selecting bazedoxifene as the SERM in this combination is that it may counterbalance estrogen stimulation of endometrial and breast tissue, without the

requirement for using a progestin in women with an intact uterus or menopausal vasomotor symptoms, while preserving or increasing BMD. (Gruber & Gruber, 2004; Lewiecki, 2007a) The combination of a SERM with conjugated estrogen has directed to a new class of menopausal therapy called "tissue selective estrogen complex (TSEC)". (Stovall & Pinkerton, 2008)

Preclinical studies have shown that bazedoxifene antagonizes estrogen-induced uterine and mammary gland stimulation more effectively than other SERMs like raloxifene and lasofoxifene. (Peano et al., 2009; Kharode et al., 2008) A randomized, double-blind, placebo-controlled Phase III trial in 3,397 postmenopausal women examined the effect of bazedoxifene 10, 20, or 40 mg combined with conjugated estrogens 0.625 mg or 0.45 mg on bone and endometrium. In this trial, the bazedoxifene plus conjugated estrogen combination therapy showed a statistically significant increase in BMD and a decrease in bone biochemical turnover markers compared with placebo. In addition to the positive effects on bone, bazedoxifene plus conjugated estrogen therapy significantly reduced the incidence and severity of hot flushes and improved vulvo-vaginal atrophy compared with placebo, with a good safety and tolerability profile. (Archer et al., 2009; Lobo et al., 2009; Pickar et al., 2009; Lindsay, 2011)

2.5.4 Safety

Miller et al., showed that deep venous thromboembolism was rare with bazedoxifene (0% to 0.6% with various doses after 2 years) and similar to placebo (0.3%). Leg cramps were similar to raloxifene and placebo. Hot flushes incidence and severity were comparable to raloxifene, but a little higher than with placebo. (Miller et al., 2008) In the study by Silverman et al, leg cramps (10.9% to 11.7% with various doses after 3 years) and deep venous thromboembolism (0.4% to 0.5% with various doses after 3 years) were significantly more common with bazedoxifene than with placebo (8.2 for leg cramps and 0.2% for deep venous thromboembolism), while breast cyst/fibrocystic breast disease was significantly less frequent. No difference between bazedoxifene and placebo were observed for myocardial infarction, strokes (ischemic or hemorraghic), or retinal vein thrombosis. (Miller et al., 2008; Silverman et al., 2008; Mitwally, 2008)

In conclusion, bazedoxifene seems to have enhanced selectivity compared to other SERMs. Preclinical and clinical studies suggest slight stimulatory effects on uterine tissue and the ability to antagonize estrogen uterine effects. In addition, it does not appear to increase hot flushes. In vitro studies suggest inhibitory effects, at the breast although no long-term clinical data is available on effects on breast cancer rates. The effect of bazedoxifene on the skeleton is similar to raloxifene, and bazedoxifene may be used just as raloxifene. The value of bazedoxifene may reside in a different risk profile than raloxifene, especially in terms of uterine safety, and bazedoxifene may consequently offer an alternative for prevention and treatment of osteoporosis.

2.6 Lasofoxifene

Lasofoxifene is potent third generation SERM, discovered through a synthetic program intended to isolate innovative molecules with good oral bioavailability and higher potency in vivo. It is a naphthalene derivative, structurally different from the first- and second-generation SERMs raloxifene, tamoxifen and clomiphene or idoxifene. Lasofoxifene has potent estrogenic and anti-estrogenic activity in vitro and in vivo, targeting any tissues that

have estrogens receptors, such as bone, uterus, breast, blood vessels, and liver. Competitive binding experiments demonstrate high affinity of the compound for both ERα and ERβ. Like other SERMs, lasofoxifene specifically binds to human ERα with high affinity and with a half-inhibitory concentration (IC50) which is similar to that seen with estradiol and consequently at least 10-fold higher than those reported for raloxifene, tamoxifen and droloxifene. Lasofoxifene also shows a high affinity for the human ERβ similar to the one of estradiol. (Gennari et al., 2010; Peterson et al., 2011; Swan et al., 2010)

Lasofoxifene has been investigated in postmenopausal women for the prevention and treatment of osteoporosis as well as for the treatment of vaginal atrophy. In a 2-year, randomized, double-blind study comprising 410 postmenopausal women, the mean change in lumbar spine BMD compared to placebo was significantly greater ($p<0.05$) with lasofoxifene 0.25 and 1.0 mg/day (3.6% and 3.9%, respectively) compared with raloxifene 60 mg (1.7%), although the results were comparable in total hip BMD. Lasofoxifene, as well as raloxifene, significantly reduced bone turnover markers and low-density lipoprotein cholesterol compared with placebo. Results have shown that treatment with lasofoxifene improves signs and symptoms of vaginal atrophy, as well as dyspareunia. (McClung et al., 2006a)

Safety and tolerability of lasofoxifene is comparable to that of raloxifene, although discontinuation rates due to adverse events are more common with lasofoxifene. In spite of these findings, evidence proves that lasofoxifene treatment may cause increased endometrial thickness compared with placebo, even though there has been no evidence of an increased risk of endometrial hyperplasia or cancer. Lasofoxifene did not get FDA approval for the treatment of vaginal atrophy. (Kulak Junior et al., 2010)

The PEARL trial, a 3-year pivotal fracture trial demonstrated that lasofoxifene increased lumbar spine and femoral neck BMD by roughly 3%. Moreover vertebral fractures were reduced by 42%, and non-vertebral fractures by 27%, with reduction in markers of bone turnover. Even though, lasofoxifene did not prevent hip fractures. (Clarke & Khosla 2009)

2.7 Bisphosphonates

2.7.1 Overview and mechanism of action

Bisphosphonates belong to a class of antiresorptive drugs, whose antifracture action is well established in randomized controlled trials. It is important to remember that a direct comparison between them has not been made, which avoids establishing a clear superiority order. There were attempts to compare them through the respective trials and the respective risk reductions, but this approach has limitations that can only be overcome with direct and randomized trials of the different drugs.

Bisphosphonates reduce the risk of fracture due to its inhibitory action of osteoclasts, which allows the osteoblasts to synthesize bone in the resorption spaces and some bone lacunae. This leads to an increase in bone mass. But, in addition, the bisphosphonates improve bone quality, by preserving the bone architecture, as shown in trials, in which the biopsies of the treated patients and controls have been studied. When the treatment with bisphosphonates is indicated, it is essential to administer calcium and vitamin D to assure its maximum antifracture efficacy. (Olmos-Martinez & Gonzalez-Macias 2008)

Farnesyl pyrophosphate synthase (FPPS) is an essential regulatory enzyme in the mevalonate pathway. This pathway is important for the production of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP), which serve as the foundation for the biosynthesis of molecules used in processes as diverse as terpenoid

synthesis, protein prenylation, cell membrane maintenance, hormones, protein anchoring, and N-glycosylation. It is also very important in steroid biosynthesis. Blocking this pathway has a variety of clinical uses, i.e. with statins for the inhibition of hydroxymethylglutaryl-CoA reductase and thus reducing cholesterol biosynthesis, and nitrogenated bisphosphonates used for osteoporosis treatment. (Kavanagh et al., 2006)

Bisphosphonates are pyrophosphate analogs in which the central oxygen has been substituted by a carbon atom and two side chains (R1 and R2). Their intestinal absorption is very low but the affinity for bone is extreme, and once there, they act as very potent antiresorptives. Two phosphate groups are essential so they can bind to bone and for the antiresorptive effect. The long side-chain (R2) determines the chemical properties, the mode of action and the strength of bisphosphonate drugs. The short side-chain (R1), principally influences pharmacokinetics and chemical properties. (Olmos-Martinez & Gonzalez-Macias 2008; Kavanagh et al., 2006)

While some of the first generation bisphosphonates such as etidronate and clodronate act by reversing pyrophosphorylytic reactions catalyzed by aminoacyl-tRNA synthetases, thus producing the corresponding pyrophosphonate analogs of adenosine tri phosphate and osteoclast apoptosis, the action of the nitrogenated bisphosphonates involves a different mechanism, inhibiting the FPPS activity in the mevalonate pathway. Their superior potency results from two main properties: the affinity for bone mineral and the ability to inhibit osteoclast function. (Olmos-Martinez & Gonzalez-Macias 2008)

The difference in potency of the different nitrogenated bisphosphonates depends on their affinity for bone and their capacity of inhibiting FPPS. Their affinity for bone tissue provide bisphosphonates with the capacity of remaining embedded in bone matrix for a long time, thus providing the possibility of weekly, monthly or even yearly regimens. As mentioned earlier, bisphosphonates are poorly absorbed by the intestine (between 1% and 3%) and consequently bioavailability can vary considerably. The new third generation bisphosphonates are administered intravenously, avoiding this difficulty and accordingly increasing the effect of these drugs. (Olmos-Martinez & Gonzalez-Macias 2008)

2.8 Etidronate

This bisphosphonate was the first one introduced into osteoporosis treatment. At the present time, it is practically not used anymore. Its biggest advantage is probably its price. It increases bone mass in the spine and femur. It reduces the incidence of vertebral fractures; however it has not proven to diminish femoral fractures. Its administration is oral and cyclic, a dose of 400 mg, once a day, during 2 weeks and repeated every 90 days. During the intervals calcium is administrated. There is only one study where its intravenous administration was compared with clodronate and placebo for a short period, and it reduced bone mass loss in the spine.

2.9 Clodronate

It has been used for postmenopausal osteoporosis treatment in oral and intravenous regimes. The studies show that it prevents bone loss in vertebral spine when comparing with controls, and it has similar effects to estrogens at 2 years. In a 6-year trial, it was observed that it not only increased bone mass, but it also reduced vertebral fractures. McCloskey et al. conducted a 3-year, double blind, placebo controlled trial to study the effect of oral clodronate (800 mg daily) in fracture prevention. In this trial clodronate was

associated with a significant increase in the mean lumbar spine and hip BMD. Moreover, it significantly reduced vertebral fracture risk (relative risk, 0.54; 95% CI, 0.37-0.80; p<0.0001). Rovetta et al. have published a study that shows that treating osteoporotic vertebral fractures with 300 mg of intravenous clodronate may have better results than paracetamol in reducing pain. In spite of these results, since the discovery of the potent nitrogen bisphosphonates, the first generation bisphosphonates have been relegated to the last line of treatment. (McCloskey et al., 2004)

2.10 Alendronate (Alendronic acid)

Alendronate is one of the bisphosphonates most widely used. It increases vertebral bone mass around 6-8% and 3-6% at the hip in postmenopausal osteoporotic women treated for 3 years. It shows a decrease of vertebral and non-vertebral fractures of about 50% in this period of time. Ninety five percent of postmenopausal women respond maintaining or increasing bone mass. Alendronate has shown to be able to prevent loss of bone mass of postmenopausal young women with osteopenia and in fragile old women living in retirement homes. In male osteoporosis, it has showed increases of 5% in bone mass at 2 years of treatment. There is reliable security data of the drug at 10 years. Alendronate is approved by the Food and Drug Administration (FDA) of the United States for the treatment of osteoporosis in men and glucocorticoid-induced osteoporosis.

It is administered orally, in weekly doses of 70 mg, fasting with 200 ml of water. The intake of food or drinks has to be avoided in the next 30 minutes and orthostatism has to be kept for this time. There is a preparation of alendronate, which has been available since 2007, that combines the drug with 2,800 U or 5,600 IU of vitamin D. Even though it is commercialized with another name, it is administered in the same way as alendronate alone and it is indicated in patients that need a supplement of vitamin D, but have an adequate intake of calcium.

The pivotal trial of alendronate, the FIT (Fracture Intervention Trial), showed that the risk of any clinical fracture was lower in the alendronate than in the placebo group (139 (13.6%) vs 183 (18.2%); relative hazard=0.72 (0.58-0.90)). The relative hazards for hip fracture and wrist fracture for alendronate versus placebo were 0.49 (0.23-0.99) and 0.52 (0.31-0.87). (Black et al. 1996) Ensrud et al. published the analysis of a sub group of patients of the FIT. These patients were patients at high risk of fracture. Their results show a 47% significant reduction in risk of new vertebral fractures in the alendronate group compared with the placebo group. The reduction in risk of new vertebral fracture was consistent across fracture risk categories including age (RR=0.49 in women < 75 years compared with 0.62 in those ≥75 years), BMD (RR=0.54 in women with a femoral neck BMD < 0.59 g/cm2 [median] compared with 0.53 in those with a BMD ≥ 0.59 g/cm2), and number of preexisting vertebral fractures (RR=0.58 in women with 1 vertebral fracture compared with 0.52 in those with ≥ 2). The overall significant 28% reduction in risk of incident clinical fractures in the alendronate group compared with the placebo group was also observed within these subgroups. (Ensrud et al., 1997) Several other publications have derived from the FIT population, studying multiple symptomatic fractures, bone mineral density, biochemical markers of formation and resorption, fracture prevention in osteopenic women, effect of alendronate continuation versus discontinuation, and effect in those women who lost bone during treatment. (Levis et al., 2002; Bauer et al., 2004; Chapurlat et al., 2005; Quandt et al., 2005)

In conclusion, alendronate is a well-tolerated, safe and effective drug for the treatment of postmenopausal osteoporosis, osteoporosis in men and glucocorticoid induced osteoporosis.

2.11 Risedronate

This drug has showed to increase bone mass in spine and hip and to significantly reduce the risk of fracture in postmenopausal women. Treatment of postmenopausal women with osteoporosis with risedronate during three years has shown to reduce vertebral fractures in approximately 50% and non-vertebral fractures in 39%. At the hip, the fracture reduction is between 40 and 60%. At 5 years, the results are similar. The drug has shown anti fracture efficacy after 6 months of administration. In other studies it has been confirmed that the efficacy still remains after 7 years of treatment with a good security profile. Risedronate has shown to be efficient in the prevention of spinal and femoral bone mass loss in patients with osteopenia. The first available preparation was 5 mg that was administered daily. A couple of years later a preparation of 35 mg became available and had to be taken weekly. Finally, about two years ago, a preparation of 150 mg came out to be taken every month. In Europe, this preparation was split into two 75 mg capsules that have to be taken on consecutive days once a month. Every dosage preparation of risedronate has to be taken following the instructions of oral bisphosphonate administration mentioned earlier.

In one of the main trials of risedronate, McClung et al, studied 5445 women 70 to 79 years old diagnosed with osteoporosis (T score at the femoral neck more than -4 SD below the mean or lower than -3 plus a non-skeletal risk factor for hip fracture, such as poor gait or a tendency to fall) and 3886 women at least 80 years old with at least one non-skeletal risk factor for hip fracture or low bone mineral density at the femoral neck (T score, lower than -4 or lower than -3 plus a hip-axis length of 11.1 cm or greater). The patients were randomly assigned to receive treatment with oral risedronate (2.5 or 5.0 mg daily) or placebo for three years. The primary end point was the incidence of hip fracture. The results showed that the incidence of hip fracture among the patients assigned to risedronate was 2.8%, as compared with 3.9% among those assigned to placebo (relative risk, 0.7; 95% CI, 0.6 to 0.9; p=0.02). In the group of women with osteoporosis (those 70 to 79 years old), the incidence of hip fracture among those assigned to risedronate was 1.9%, as compared with 3.2% among those assigned to placebo (relative risk, 0.6; 95% CI, 0.4 to 0.9; p=0.009). In the group of women selected primarily on the basis of non-skeletal risk factors (those at least 80 years of age), the incidence of hip fracture was 4.2% among those assigned to risedronate and 5.1% among those assigned to placebo (p=0.35). (McClung et al., 2001)

To evaluate vertebral fracture risk reduction, Reginster et al, completed a randomized, double-blind, placebo-controlled study to determine the efficacy and safety of risedronate in the prevention of vertebral fractures in postmenopausal women with established osteoporosis. The study was conducted at 80 study centers in Europe and Australia. A total of 1226 postmenopausal women with two or more prevalent vertebral fractures received risedronate 2.5 mg or 5 mg daily or placebo. The study lasted 3 years; however, the 2.5 mg group was discontinued by protocol amendment after 2 years. Lateral spinal radiographs were taken annually for evaluation of vertebral fractures, and BMD was measured every 6 months. Risedronate 5 mg reduced the risk of new vertebral fractures by 49% over 3 years compared with control ($p<0.001$). A significant reduction of 61% was observed within the first year ($p = 0.001$). The fracture reduction was similar in both groups at 2 years. The non-vertebral fracture risk was reduced by 33% compared with control over 3 years ($p = 0.06$). Risedronate significantly increased BMD at the spine and hip within 6 months. In conclusion, risedronate 5 mg was an effective and well-tolerated therapy for severe postmenopausal osteoporosis, reducing the incidence of vertebral fractures and improving bone density in women with established disease. (Reginster et al., 2000)

2.12 Ibandronate

Its absorption is similar to the one of the rest of the oral bisphosphonates since only 0,6% of the administrated dose is absorbed. The administration instructions are also the same as the other oral bisphosphonates, because if it is administered concomitantly with food, the plasmatic concentrations can decrease up to 90%. In studies at 3 years, it has shown to reduce vertebral fractures (52%) and increase vertebral BMD (6.5%) without presenting significant adverse effects or changes in bone histology. It also avoids bone loss in postmenopausal women with osteopenia and it has proven to be very efficient in preventing bone loss in Glucocorticoid-induced osteoporosis. In women with severe osteoporosis (T score <-3), it reduces non-vertebral fractures up to 69%. (Chesnut, 2006)

It is administered orally in a monthly dose of 150 mg and also intravenously every three months in a dose of 2 mg. Randomized clinical trials like MOPS (Monthly Oral Pilot Study) or MOBILE (Monthly Oral Ibandronate in Ladies) have shown that the ibandronate monthly dose is as effective and secure as the daily dose. In the general population of the pivotal trial (BONE, Oral Ibandronate Osteoporosis Vertebral Fracture Trial in North America and Europe), the frequency of adverse events of the gastrointestinal tract in the daily dose and the intermittent dose was comparable to the one of placebo. Dyspepsia was the only adverse event with a slight superior frequency in patients in active treatment with ibandronate. (Delmas et al., 2004)

In different chronic therapeutical areas it has been shown that, for oral bisphosphonates, the treatment compliance is poor and, besides, it decreases with time. This has a big impact on the drug's effectiveness, since the early interruption and bad compliance decrease significantly the benefit that these drugs could have. In order to show the importance of the problem, a sub study, with data of the IMPACT study was made. More than 2,300 postmenopausal women were treated for osteoporosis with risedronate. The analysis showed, that, in contrast to the women who did not accomplish the treatment, most of the women, who complied with the treatment, had decreases in the bone resorption markers. In other analysis, that included patients with osteoporosis and osteopenia from a Canadian data base, it was shown that patients with a treatment compliance higher than above 80% had increases in bone mineral density and these were significantly higher than the ones from patients who did not accomplish this requirement. The strict administration requirements that are needed caused some patients to interrupt the treatment with bisphosphonates and it was a reason for some not to start it. In a 6-month study with alendronate, 14.3% of the patients mentioned discomfort as the reason for interrupting the treatment. In prospective trials of crossover treatment it has been shown that the least frequent administration of bisphosphonates increases the treatment compliance. That is why the use of ibandronate with monthly intake could be of benefit. (Delmas et al., 2007) On the other hand, ibandronate has not shown, in randomized controlled trials, reductions in the incidence of non-vertebral or hip fractures.

2.13 Zoledronate (Zoledronic acid)

Zoledronic acid is a third generation bisphosphonate. Its complete chemical name is 1-hydroxi-2-(1H-imidazol-1-y-1)ethylidene) bisphosphonic acid. The experience with this drug is more extensive in the oncology area. Besides oncology, zoledronic acid has other non-oncological indications like postmenopausal osteoporosis treatment, established osteoporosis treatment, glucocorticoid induced osteoporosis, male osteoporosis and Paget's

disease of the bone. The difference is not only in the indication, since the administration regime is also different.

Zoledronate is approximately 2-3 times more potent than pamidronate, it is more or less as potent as alendronate, risedronate and ibandronate, but when it is administrated intravenously, the gastrointestinal adverse effects are avoided and the bioavailability increases, while at the same time, it increases the compliance to 100%. The pharmacokinetics of the drug is very similar to that of the other bisphosphonates. The highest plasmatic concentration is reached just after the infusion, with a posterior descent of approximately 10% in 4 h, followed by 1% in the next 24h. The mean urine excretion of the drug is around 44% of the administered dose, which means that the bone tissue absorbs more than 50% from the administered zoledronate.

The HORIZON study (Health Outcomes and Reduced Incidence with Zoledonic Acid Once Yearly Pivotal Fracture Trial) is a multicentric, international, double blind, placebo-controlled trial of postmenopausal women with osteoporosis, whose objective was to show superiority of 5mg of intravenous zoledronic acid against placebo administrated during a period, not shorter than 15 min. In this study, as in most osteoporosis studies, patients received 1.000-1.500 mg of calcium and 400-1.200 U of vitamin D. The patients had densitometric osteoporosis or densitometric osteopenia with at least 2 mild to moderate vertebral fractures. (Lyles et al., 2007) Finally, more than 7.700 patients took part in the study and were followed for 3 years; paying special attention to new fractures, bone remodeling biochemical markers and densitometric changes. At the end of the study, the patients that had received zoledronic acid showed a reduction in the vertebral fracture risk of 70%. The reduction was similar for the first and second year of the study, ranging from 60 to 71%. Besides, patients treated with zoledronate showed a reduction of 41% in hip fracture risk and 25% in non-vertebral fracture risk. The results of bone density and biochemical bone remodeling markers were also significantly better for the group of patients treated with zoledronic acid. Moreover, the increase in bone mineral density was over 6% in the lumbar spine and total hip, and over 5% in the femoral neck. The biochemical markers of bone remodeling, after the first infusion of zoledronic acid, experienced an important decrease, as expected, and they remained stable during the whole study. (Black et al., 2007)

Many patients showed adverse effects during the study, being more frequent in the treatment group. The difference was basically the post-infusion syndrome. This syndrome appeared usually 24-48 hours after the zoledronic acid infusion, just as described by other intravenous bisfosfonates, it happens sometimes, even with the ones administrated orally, and it disappears on the third day post-infusion. The symptoms are light fever, myalgias, flu-like syndrome, headache and/or arthralgias and they disappear with analgesic, non-steroid anti-inflammatory drugs or paracetamol. The episode appears usually after the first infusion and in seldom cases, after the second one. It shows an incidence with a clear descendent pattern in the later infusions. Some of the patients showed transient renal function deterioration from 9 to 11 days after the infusion, however it did not have any clinical transcendence. (Black et al., 2007)

Probably the most important finding related to the treatment with zoledronate would be the 28% decrease in mortality independent of the cause, showed in a population of over 2.000 patients with femur fracture. (Lyles et al., 2007)

In conclusion, zoledronate treatment is very efficient for the decrease of vertebral, non-vertebral and hip fractures. It decreases mortality for any cause after a femur fracture.

Besides, it is a secure treatment that eludes the gastrointestinal adverse events and the bad adherence that are usually seen with other bisfosfonates, but it has to be remembered that, due to its administration route, zoledronate is not for ambulatory use and it has to be administrated very carefully in patients with chronic renal impairment, and those who need to get dental extractions.

2.14 Cost effectiveness

Economical evaluation in medicine includes different types of studies that enable us to give the population better care and attention with resources that are limited. Among the many different types of analysis, we can find cost reduction assays, analysis of cost-effectiveness (mostly used), analysis cost-utility and cost-benefit. The cost-effectiveness analysis is probably the easiest to evaluate, since it expresses the monetary units needed to change the units normally used in clinical practice (viral charge, number of fractures avoided or quality adjusted life years, QALY). (Sacristan et al., 2004a, Sacristan et al., 2004b)

Internationally a sanitary intervention is considered to be cost-effective if the additional cost for QALY gained, in comparison with another intervention, is below the 50.000.00 US$ and it is not when it is higher than 100.000.00 US$. In Spain, the amount used is € 30.000.00 in order to validate the acceptance of interventions. However, there are many authors, who consider this value to be excessively low. (Sacristan et al., 2004a, Sacristan et al., 2004b)

In a review of cost-effectiveness analysis, in which 23 studies were included, and some of which included more than 90 clinical trials, it was demonstrated that the available bisfosfonates in Spain show that the cost of risedronate, compared with no treatment in women with previous fracture is of € 43.601.00 and for alendronate € 49,483.00. In women without previous fracture the values increase to € 61,604.00 for risedronate and € 88,634.00 for alendronate, both compared with patients not treated. If we consider only patients over 65 years old, treatment with alendronate as well as with risedronate result cost-effective in patients with previous fracture as well as in patients with no previous fracture. As expected, the bigger the population is, the more cost effective is treatment with bisphosphonates. (Van Staa et al., 2007; Fleurence et al., 2007)

It is important to consider that this data is subject to several conditions, such as the comparator and the sample used during the clinical trials, but most importantly the price of the drug. The data used in the Spanish studies presented previously are from 1999, which differ from the actual reality. There are other studies that analyze new bisfosfonates as ibandronate and zoledronate, but not for osteoporosis.

2.15 Security of bisphosphonates

These drugs are usually well tolerated, as long as they are taken in a scrupulous way and the intake instructions are followed. Esophageal ulcerations have been known to occur when the drugs are administrated orally and daily. In spite of the good profile that its weekly administration has, it should not be administered to individuals with gastric ulceration or esophageal ulceration, or to those who present pyrosis (heartburn) and require medication. They should not be administrated to pregnant women, or to patients with severe renal function impairment. The intravenous bisphosphonates usually produce acute phase reactions with fever, arthromyalgia and flu like syndrome, that usually disappear by the second administration and that can be relieved with the concomitant use of paracetamol or ibuprofen. Hypocalcaemia can appear more often; therefore it is wise to use calcium and

vitamin D concomitantly. The renal function has to be controlled before and after the administration of intravenous bisphosphonates.
Avascular necrosis of the jaw, also called osteonecrosis of the jaw is an illness that has worried many physicians, ever since Marx described it for the first time in 2003 and it will be described in detail in another chapter. (Marx, 2003)

2.16 Long-term effects of the treatment with bisphosphonates: Atypical hip fractures

Reports associating atypical fractures of the femur with long-term use of bisphosphonates led the initiative of the American Society for Bone and Mineral Research (ASBMR) to form a task force to address key questions related to this finding. The task force defined major and minor features of incomplete and complete atypical femoral fractures and recommended that all major features, including their location in the subtrochanteric region and femoral shaft, transverse or short oblique orientation, minimal or no associated trauma, a medial spike when the fracture is complete, and absence of comminution, be present to appoint a femoral fracture as atypical. Minor features include their relationship with cortical thickening, a periosteal reaction of the lateral cortex, prodromal pain, bilaterality, delayed healing, co-morbid conditions, and concomitant drug use, including bisphosphonates, other antiresorptive agents, glucocorticoids, and proton pump inhibitors. Preclinical data evaluating the effects of bisphosphonates on collagen cross-linking and maturation, accumulation of micro-damage and advanced glycation end products, mineralization, remodeling, vascularity, and angiogenesis provide biologic plausibility to a potential association with long-term use of bisphosphonates. Based on published and unpublished data and the extensive use of bisphosphonates, the incidence of atypical femoral fractures associated with bisphosphonate use for osteoporosis appears to be very low, particularly compared with the number of vertebral, hip, and other fractures that are prevented. Furthermore, a causal association between bisphosphonates and atypical fractures has not been established. However, recent observations imply that the risk rises with increasing treatment duration, and there is concern that lack of knowledge and underreporting may mask the real incidence of the problem. Given the relative infrequency of atypical femoral fractures, the task force recommends that specific diagnostic and procedural codes be created and that an international registry be established to assist studies of the clinical risk factors and optimal surgical and medical management of these fractures. Physicians should be made aware of the possibility of atypical femoral fractures through a change in labeling of bisphosphonates. (Shane et al., 2010)
A study comprising 12,777 Swedish women 55 years of age or older, who sustained a fracture of the femur in 2008 was published recently. Radiographs of 1,234 of the 1,271 women with a subtrochanteric or shaft fracture were reviewed. Fifty-nine patients with atypical fractures were identified. The relative and absolute risk of atypical fractures associated with bisphosphonate use was estimated by means of a nationwide cohort analysis. The 59 case patients were also compared with 263 control patients who had typical subtrochanteric or shaft fractures. The cohort analysis showed an age-adjusted relative risk of atypical fracture of 47.3. The increase in absolute risk was 5 cases per 10,000 patient-years. A total of 78% of the fractured patients and 10% of the controls had received bisphosphonates (multivariable-adjusted odds ratio of 33.3). The risk was independent of coexisting conditions. After drug withdrawal, the risk diminished by 70% per year since the last use (odds ratio, 0.28; 95% CI, 0.21 to 0.38). (Schilcher et al., 2011)

2.17 Biological agents

The illnesses that provoke bone loss, like osteoporosis, derive from the imbalance in the cycles of bone remodeling favoring bone resorption. The receptor activator of the nuclear factor kB (RANK), a member of the tumor necrosis factor (TNF) family proteins, and its ligand (RANKL) are fundamental for differentiation, activation and survival of osteoclasts and, therefore, basic mediators of the regulation of bone remodeling. (Anderson et al., 1997; Burgess et al., 1999; Lacey et al., 1998) It has been demonstrated that the signaling of the RANKL is involved in the pathophysiology of many bone loss illnesses, such as primary and many secondary osteoporoses. RANKL production is increased when estrogen is decreased. (Eghbali-Fatourechi et al., 2003) This condition occurs in menopause and in circumstances of hormonal ablation, and leads to an increase in bone resorption. In animal studies with knockout mice lacking RANKL, an absence of osteoclast can be seen, and consequently an increase in bone density. (Kong et al., 1999)

2.18 Denosumab

Denosumab, a fully human monoclonal IgG2 antibody to RANKL imitates the effects of osteoprotegerine (OPG), endogenous inhibitor of RANKL blocking bone resorption. Denosumab presents a much longer half-life and it is highly specific since it does not bind to other members of the TNF family, including TNF, TNF-related apoptosis-inducing ligand, or CD40 ligand. (Bone et al., 2008; Kearns et al., 2008; Kostenuik et al., 2009) The binding of denosumab to RANKL prevents rank activation and inhibits the formation, activation and survival of osteoclasts.

Comercial denosumab comes as a sterile, colorless solution intended for subcutaneous injection. It comes ready for administration in a 60mg/ml syringe-vial. The prefilled syringe drug product contains denosumab at 60 mg/mL, 17 mM sodium acetate, 4.7% sorbitol, and 0.01% polysorbate 20, at a pH of 5.2, filled to a target deliverable volume of 1.0 mL.

2.18.1 Pharmacodynamics and pharmacokinetics

The pharmacodynamic profile of denosumab appeared alike across all the subject populations studied. So far it has been studied in healthy postmenopausal women (including a Japanese population), healthy men ≥ 50 years of age, subjects with advanced cancer and bone metastases (breast cancer, other solid tumors [excluding lung], and multiple myeloma), and subjects with rheumatoid arthritis. The results indicate that SC administration of 60 mg denosumab causes a quick reduction in bone resorption within 6 hours, assessed by the marker C-telopeptide of type 1 collagen (CTX1) in serum (approximately 70% reduction), with an approximately 85% reduction occurring by 3 days.

Serum CTX1 reductions were maintained for 6 months after the 60-mg dose, with the serum CTX1 reductions partially attenuated from a maximal reduction of ≥ 87% to reductions of approximately 45% or greater (range 45% to 80%), reflecting the reversibility of its effects on bone remodeling. The pharmacokinetics following IV or SC administration of denosumab has been studied at doses up to 3 mg/kg or 210 mg in various populations, including all those described earlier. Following subcutaneous administration, denosumab exhibits dose dependent, nonlinear pharmacokinetics over a wide dose range (as observed for other monoclonal antibodies). Nevertheless, dose-proportional increases in exposure were observed for doses ≥ 60 mg, consistent with saturable and non-saturable mechanisms of elimination. Its bioavailability is approximately 60% after SC injection. No accumulation in

serum denosumab concentrations was observed with repeated doses of 60 mg every 6 months. There is no evidence that the rare (approximately 0.5% of treated subjects) and transient development of binding antibodies to denosumab influences its pharmacokinetics or pharmacodynamics. Changes in serum calcium levels following administration of denosumab are not related to the extent of exposure. (Yonemori et al., 2008; Perez-Edo, 2011)

2.18.2 Denosumab in human clinical trials

Information is available from 44 clinical trials in healthy adults and patients with osteoporosis (approximately 13,500 subjects), bone loss associated with hormone-ablation therapy (approximately 1,900 subjects), rheumatoid arthritis (approximately 200 subjects), advanced cancer (multiple myeloma and advanced malignancies involving bone [approximately 7,800 subjects]) and giant cell tumor of the bone (approximately 260 subjects) collected between June 2001 to November 2010.

In the *Denosumab Fortifies Bone Density* (DEFEND) trial, a phase III randomized, placebo controlled study, of 332 postmenopausal women with osteopenia and stratified according to the beginning of menopause (<5 years, >5 years), denosumab demonstrated a significant increase in lumbar BMD (6.5%) at 24 months, compared with placebo (-0.6%). It also increased BMD in other locations as total hip, distal third of the radius, and whole body ($p > 0.001$) in the two patients' strata. The incidence of adverse effects was similar between the placebo group and the denosumab group. (Bone et al., 2008)

In a comparative clinical trial, the DECIDE (*Determining Efficacy: Comparison of Initiating Denosumab vs. Alendronate*) trial, comprising 1,189 postmenopausal women with low BMD (T-score: ≤-2 SD), patients were randomized 1:1 to receive subcutaneous denosumab (60 mg every 6 months) plus oral alendronate placebo weekly or oral alendronate weekly (70 mg) plus a subcutaneous denosumab placebo injection every 6 months. Denosumab increased total hip BMD when compared to alendronate (3.5 % vs. 2.5 %, $p < 0.00001$). A greater increase in BMD could be seen with denosumab than with alendronate in other locations, as in the trochanter (4.5 % vs. 3.5 %), distal radius (1.1 % vs. 0.6 %), lumbar spine (5.3 % vs. 4.2 %) and femoral neck (2.2 % vs. 1.6 %); $p < 0.0003$. The safety profile was similar for the two groups. No patient included in the study developed antibodies against denosumab. (Brown et al., 2009)

Another phase III, multicenter, double blind study, called STAND (*Study of transitioning from Alendronate to Denosumab*) was performed to evaluate the effect of denosumab in patients who were receiving alendronate. Five hundred four postmenopausal women ≥ 55 years of age with a BMD T-score of <-2.0 and >-4 SD, who were receiving weekly oral alendronate for at least 6 months, were randomized and treated for 44±33 months. Changes in BMD and bone biochemical markers were evaluated. At 12 months the group receiving denosumab (but previously treated with alendronate) showed a significantly higher increase in total hip BMD compared to those still receiving alendronate (1.9% vs. 1.05%; $p < 0.00012$). Significantly higher BMD increases with denosumab compared with alendronate were also seen at 12 months at the lumbar spine, femoral neck, and distal radius (all $p < 0.0125$). The adverse events and serious adverse events were similar in both groups of treatment. (Kendler et al., 2010)

Finally, the main phase III trial, the FREEDOM (*Fracture Reduction Evaluation of Denosumab in Osteoporosis every 6 Months*) trial, comprised 7,868 postmenopausal women with osteoporosis with a BMD T-score between <-2.0 and >-4 SD and evaluated the efficacy in

fracture reduction of denosumab. The patients received 60 mg subcutaneous denosumab or placebo every 6 months for 36 months. Around 23% of the patients had a previous vertebral fracture. The patients' retention rate in the study was 83%. The new fracture relative risk reduction was 68% (2.3% vs. 7.2%; $p<0.0001$) for vertebral fractures, 20% (6.5% vs. 8.0%) for non-vertebral fractures and 40% (0.7% vs. 1.2%) for hip fractures. As compared with subjects in the placebo group, subjects in the denosumab group had a relative increase of 9.2% in bone mineral density at the lumbar spine and 6.0% at the total hip at 36 months. There were no significant differences between subjects who received denosumab and those who received placebo in the total incidence of adverse events, serious adverse events, or discontinuation of study treatment because of adverse events. No cases of osteonecrosis of the jaw were observed in either group. (Cummings et al. 2009)
In conclusion, denosumab offers a highly effective alternative to the treatment of osteoporosis by decreasing bone resorption and increasing bone mineral density through the inhibition of RANKL.

2.18.3 Cost effectiveness studies of the treatment with denosumab

One of the clear advantages of denosumab is its administration route and dosage. A subcutaneous injection every 6 months is very comfortable and increases the adherence to treatment. This finding is very important when establishing the cost-effectiveness advantages of any treatment since fracture prevention is improved when adherence is optimal. In order to establish the cost-effectiveness of denosumab compared to generic alendronate, branded risedronate, strontium ranelate and no treatment in a Swedish setting, Jönsson et al, designed a Markov cohort model and followed them for 5 years. The mean age of the Typical Swedish patient population is 71 years old, with a mean BMD T-score of ≤ 2.5 SD and a prevalence of morphometric vertebral fractures of 34%. Treatment persistence and residual effect after discontinuation was assumed to be equal to the time on treatment. Persistence with the comparators and with denosumab was derived from prescription data and a persistence study, respectively. The base-case incremental cost-effectiveness ratios were anticipated at €27,000, €12,000, €5,000, and €14,000, for denosumab compared with generic alendronate, risedronate, strontium ranelate, and no treatment, respectively. Fracture and unit costs, as well as mortality rates for the general population were based on data from 2008. Suboptimal persistence had the greatest impact in the comparison with generic alendronate, where the difference in drug cost was larger. They concluded that persistence improvement impacts positively on cost-effectiveness increasing the number of fractures prevented in the population targeted for osteoporosis treatment. (Jonsson et al., 2011) In another similar study, denosumab was cost effective compared with all other therapies. In particular, denosumab was found to be cost effective compared with branded alendronate and risedronate at a threshold value of €30,000 per QALY and denosumab was dominant (lower cost and greater effectiveness) compared with risedronate from the age of 70 years in women with a T-score of -2.5 or less and no prior fractures. (Hiligsmann & Reginster, 2011)

3. Anabolic agents

3.1 Fluoride

Fluoride is the anion F-, a monovalent ion (-1 charge). At high doses it can be lethal to humans. At low doses, 1 to 2 mg per day, it prevents dental caries. At intermediate doses,

ranging from 8 to 80 mg daily, skeletal fluorosis can develop. These doses are not so rare, they can be found in some regions with high fluoride levels in well waters or in some industrial settings. It was thought to be a therapeutical agent after observing the osteosclerosis effect at high doses. (Heaney, 1994)
Many years ago, endemic fluorosis was described in patients living in regions with high fluoride water levels or grounds where vegetables and tea were cultivated, like some places in India. Moreover some observations were published describing a low fracture incidence in patients living in areas with high fluoride levels. It was used for the treatment of osteoporosis for the first time in 1961. It was approved for the use of osteoporosis prevention in several countries in Europe, but it never got the approval from the American Federal Drug Administration (FDA). (Pandey & Pandey 2011; Turner, 1996)

3.1.1 Pharmacokinetics

Two different types of compounds have been used for the treatment of osteoporosis: sodium fluoride and monofluorophosphate. Sodium fluoride could be found as capsules or tablets with an enteric protection. A more recent preparation is the sustained release formulation. The ion equivalences usually used were: monofluorophosphate 200 mg containing 16,4 mg of fluoride and sodium fluoride 50 mg containing 22,6 mg if the ion. (Watts, 1999)
Absorption and bioavailability of fluoride preparations depend on their formulation. Thus sodium fluoride is quickly absorbed in the stomach with maximum plasma fluoride levels 30 minutes after ingestion with almost a 100% bioavailability, while sodium monofluorophosphate absorption is slower with a bioavailability around 65%. It is cleared by the kidney and about 50% of absorbed fluoride is deposited in the skeleton. Its distribution is not homogeneous since higher amounts are deposited in areas with high bone remodeling rate, such as trabecular bone. (Ekstrand & Spak, 1990)

3.1.2 Mechanism of action

Fluoride increases bone formation increasing osteoblasts proliferation, without altering their differentiation. The molecular basis of this mitogenic action is not known, but there are several hypotheses. The most popular one indicates that fluoride induces an increase in tyrosine phosphorilation of signaling proteins of the mitogenic process. Besides this effect over the osteoblastic cells, fluoride modifies the crystallization of the bone tissue. Thus replacing hydroxylic radicals of hydroxyapatite, forming fluoroapatite, a compound with a lesser structural stability and more resistance to osteoclastic resorption. (Marie et al., 1992)

3.1.3 Effect of fluoride in bone mineral density and fracture risk reduction

Results from studies evaluating the effect of fluoride compounds on BMD agree that this agent increases lumbar spine BMD in a consistent and linear manner. The increases in spinal BMD vary between 2.3 and 9% annually. A recent meta-analysis establishes the mean increase of spinal BMD in 8.1% at two years and 16.1% at four years, when comparing it to placebo. The increase in BMD depends on the doses, formulation and fluoride compound used. An interesting finding among all the trials is that there are 25% of patients considered as non-responders, since they did not experience any change in BMD during the fluoride treatment. (Heaney, 1994; Haguenauer et al., 2000a)
The results of the trials that have studied the effect of fluoride in fracture risk reduction are inconclusive. Some studies have demonstrated the decrease in the incidence of vertebral

fractures with monofluorophosphate, (Reginster et al., 1998) or sodium fluoride treatment,(Farrerons et al., 1997) while other studies, using the same preparations and doses, failed in doing so. Vestergaard et al, in 2008 published a meta-analysis including 25 different studies and concluding that in spite of the BMD increase in spine and femur, fluoride treatment did not reduce significantly vertebral risk of fracture. (Vestergaard et al., 2008) Moreover, another meta-analysis goes even further and establishes the increase in fracture risk with increasing doses at four years. (Haguenauer et al., 2000a; Haguenauer et al., 2000b)

3.1.4 Toxicity

The adverse events seen with fluoride treatment are varied. The most frequent ones are gastrointestinal symptoms and acute lower extremities pain. The frequency and intensity of these effects is dose and preparation dependant. Gastrointestinal symptoms include dyspepsia, epigastralgia, nausea and vomiting, and they appear in 10% to 40% of patients. Lower extremity pain is quite common also, appearing in around 15% of patients, especially in those patients receiving high doses of sodium fluoride or monofluorophosphate. Some authors have established a relationship between these pains and development of stress fractures. (O'Duffy et al., 1986)

Finally, bone biopsies have demonstrated that patients treated with fluoride develop an abnormal bone, consistent with an increase of trabecular width, volume and trabecular surface covered with osteoid material which can be seen inside mineralized bone. When analyzing dynamic histomorphometric indices, a reduction in tetracycline labeling and an extension in the mineralization interval can be seen. Both findings indicate a mineralization defect. (Lundy et al., 1995)

3.2 Parathyroid hormone and analogs

Among the many therapeutic options teriparatide or recombinant human PTH (1-34), occupies an important place. It is classified into a group of anabolic bone-forming drugs as opposed to the anti-resorptive or catabolic. Teriparatide is given as daily subcutaneous self-administered injections.

It induces *de novo* bone formation by increasing the rate of bone turnover in favor of formation. The treatment with teriparatide increases trabecular connectivity and cortical bone thickness. (Dempster et al., 2001) Teriparatide improves bone mechanical properties resulting in a significant decrease in vertebral and non-vertebral fractures in postmenopausal women with osteoporosis, male osteoporosis and corticosteroid-induced osteoporosis. (Keaveny et al., 2007) That is why its use is considered appropriate mainly in patients at high risk of fracture and in those who have failed previous treatments. (Hodsman et al., 2005)

The fundamental physiological action of parathyroid hormone (PTH) is the maintenance of calcium homeostasis to maintain nearly constant concentrations through the tubular resorption of calcium by stimulating calcium absorption in the bowel by vitamin D, increasing renal 1-α hydroxylase.

The effect exerted by PTH on the skeleton is complex. High levels of PTH observed in primary and secondary hyperparathyroidism, leading to increased bone resorption by its action on osteoclasts, produce secondary osteoporosis. In contrast, low levels increase the osteoblastic activity of bone formation. This would contrast with the desired effect by

administering PTH as a treatment for osteoporosis. However, it was observed that the action of the hormone on bone varies if administration is continuous (emulating persistently high levels of hyperparathyroidism) that induce catabolic effects on bone, or intermittent (as given in treatment) which also increases resorption as well as formation of bone.

3.3 Teriparatide (1-34 parathohormone)

The first available indication for teriparatide was the treatment of established osteoporosis in postmenopausal women. Of the various existing studies on this drug, the FPT (Fracture Prevention Trial) is the most important. It compared teriparatide at doses of 20 or 40 μg/day versus placebo in 1637 postmenopausal women with vertebral fractures. Patients receiving teriparatide achieved significant reductions in the rate of new vertebral and non-vertebral fractures. They also produced an increase in lumbar and femoral neck bone density. Although 40 μg/day achieved greater effects on BMD, fracture risk was not significantly different between the two doses, while the higher dose was less tolerated (11% of withdrawals due to adverse effects with 40 μg/day versus 6% with 20 μg/day or placebo). The dose of 20 μg/day showed a reduction in vertebral fracture risk of 65% and a non-hip non-vertebral fracture risk reduction of 35%. This study was initially planned to last for 36 months, but it was stopped when patients had completed an average of 21 months for security measures due to osteosarcomas observed in drug toxicity studies in rats. (Neer et al., 2001) However, in other studies it became clear that this finding occurred only in young rats treated with high doses of PTH. (Vahle et al., 2004) Moreover, no cases have been reported in humans.

A subgroup of patients were followed for up to 18 months after cessation of treatment. The subgroup of women who had received teriparatide showed a persistent 40% reduction in vertebral fracture risk at 18 months compared with placebo. These results suggest that the benefit on the incidence of non-vertebral fractures persist once it has been stopped. (Lindsay et al. 2004)

3.3.1 Combination therapy: Teriparatide plus antiresorptives

Although currently bisphosphonates are the gold standard in the treatment of osteoporosis, there are several trials that have evaluated if the association of teriparatide and BP has any beneficial effect. The studies suggest that if both drugs are administered simultaneously, bisphosphonates do not enhance, but on the contrary, seem to blunt the anabolic effect of teriparatide.

Finkelstein et al. also carried out a study in men with three groups, receiving PTH (1-34), alendronate or the combination of both. In this last group, PTH (1-34) was started at month six. All three groups were followed for 30 months. Spine BMD as measured by DXA and quantitative computed tomography. BMD was increased to a greater extent in the PTH group than in the alendronate or the combination group. Thus, these studies show no evidence of synergy between PTH and alendronate. Furthermore, alendronate may impair the anabolic activity of PTH. It is hypothesized that PTH is less effective when bone turnover is suppressed. (Finkelstein et al. 2003)

3.3.2 Teriparatide in patients previously treated with antiresorptives

Once the antagonistic effect of antiresorptives and teriparatide was observed a study was conducted to evaluate the response of teriparatide in patients previously treated with

antiresorptives. The EUROFORS study was a prospective, open-label, randomized trial of 865 postmenopausal women with established osteoporosis and was designed to investigate various sequential treatments of teriparatide over 24 months. Patients were classified into various groups depending on their previous treatments. The results of the BMD changes and biochemical markers of bone formation showed that treatment with teriparatide induces positive effects on bone mass and osteoblast function regardless of previous long-term exposure to antiresorptive therapies in postmenopausal women with established osteoporosis.

Duration of antiresorptive therapy and elapsed time between stopping previous therapy and starting teriparatide did not affect the BMD response at any skeletal site. The skeletal responses at the lumbar spine were similar among previous antiresorptive therapy groups at each time point during the study, although previous users of etidronate showed a higher increase, probably reflecting its weaker anti-remodeling activity. At month 6, total hip and femoral neck BMD significantly decreased in the previous alendronate subgroup, and total hip BMD significantly decreased in the previous risedronate subgroup. Total hip and femoral neck BMD was numerically decreased from baseline in all other subgroups at 6 months. However, this transient decrease was reversed with longer teriparatide treatment. All subgroups showed a statistically significant increase in BMD compared with baseline after 18 and 24 months of treatment, and without differences between the groups at any time point in the study. (Obermayer-Pietsch et al., 2008)

3.3.3 Sequential therapy

In another non-randomized study, 59 postmenopausal women with osteoporosis previously treated with raloxifene or alendronate for 18-36 months, were given 18-month treatment with teriparatide. Changes in BMD and bone-turnover markers were assessed. Women who had previously been treated with alendronate had a late increase in bone-turnover markers with values lower than one third those of patients who had previously received raloxifene. During the first 6 months there were significant differences in the increase in BMD at the lumbar spine and hip. Women previously treated with raloxifene had a greater increase in BMD at the two locations. At 18 months of treatment in the lumbar spine significant differences remained in favor of prior treatment with raloxifene, but in the hip differences were not significant. This demonstrates that treatment with teriparatide increases bone turnover in patients previously treated with raloxifene or alendronate, but this increase is greater and earlier in raloxifene pretreatment group. (Ettinger et al., 2004)

3.3.4 Corticosteroid-induced osteoporosis and male osteoporosis

There are also trials showing efficacy of teriparatide in the treatment of glucocorticoid-induced osteoporosis. In a randomized, double blind trial, 428 patients of both sexes aged between 22 and 89 years who had received corticosteroids for at least 3 months were randomized to receive alendronate 10mg/day or 20 μg/day of teriparatide for 18 months. After 12 months the total femur BMD was higher in the teriparatide group and at the end of study there were less vertebral fractures in the teriparatide group. (Saag et al., 2009)

Teriparatide has also been used in men with osteoporosis. The study compared men with idiopathic or secondary osteoporosis receiving teriparatide vs. placebo. The study showed increases independently of gonadal status and other factors in the teriparatide group. (Orwoll et al., 2003)

3.3.5 Side effects

In general, teriparatide, recombinant human PTH (1-34), injections are well tolerated. It is cleared from the circulation within four hours of subcutaneous administration. A daily injection is necessary and transient redness at the injection site has been noted. Headache and nausea occur in less than 10% of subjects receiving a daily dose of 20 μg Mild, early, and transient hypercalcemia can occur, but severe hypercalcemia is rare. Increases in urinary calcium (by 30 μg per day) and serum uric acid concentrations (by 13%) are seen but do not appear to have clinical consequences.

In conclusion, teriparatide is a suitable and efficient treatment option for osteoporosis. It is effective in several clinical problems, such as male osteoporosis or corticosteroids induced osteoporosis.

3.4 1-84 Parathormone

Intact PTH (PTH 1-84) has been described as having a positive effect on bone micro architecture and a reduction in the risk of new fractures due to a bone-forming mechanism. (Rosen & Bilezikian, 2001)

1-84 PTH (as well as 1-34 PTH) acts through its receptor, exerts its action in bone through osteoblasts by modulating the levels of cAMP by activating secondary messengers it acts on the osteoclast bone resorptive process. In collagen tissue, PTH in high and sustained doses inhibits its synthesis, but at low doses and used intermittently, through the action of IGF-1, it stimulates its synthesis.

PTH also increases the local synthesis of IGF-1, which may explain its anabolic effect in bone tissue. Other actions of PTH include modulation of TGF B1 and the production of prostaglandins that may contribute to bone formation, acting on the pre-osteoblast differentiation stage. And it is through the IGF-1 that it inhibits apoptosis.

This mechanism distinguishes the effect of treatment with PTH of other treatments that inhibit the resorption stage of bone remodeling acting on osteoclast (like bisphosphonates). The ability of PTH to act directly on the osteoblast, the cell that directly produces new bone, drives in the enhancement of production of new bone with consequent gain in bone mineral density and fracture risk reduction.

3.4.1 Clinical use of 1-84 PTH

Hodsman et al. conducted a study in 217 postmenopausal women with osteoporosis, with a mean age of 64.5 years, who were randomly classified into different groups receiving placebo or PTH 1-84 at doses of 50, 75, or 100 mcg. The primary endpoint was change in BMD at the lumbar spine after 1 year of treatment. The results showed a mean increase in BMD of 3.0%, 5.1% and 7.8% in the group receiving 50, 75 and 100mcg/day of 1-84 PTH respectively, compared with placebo, with all increases clearly statistically significant, whereas in the control group receiving calcium and vitamin D, there was an increase of 0.9% that did not reach statistical significance. The increase in BMD obtained by the group that received 100 mcg was statistically significant with respect to the other two groups receiving PTH, passing from a T-score of -3.2 at baseline to -2.8 at the end of treatment. In contrast there were no statistically significant differences in BMD at the hip. (Hodsman et al., 2003)

The pivotal clinical trial with 1-84 PTH is the TOP (Treatment of Osteoporosis) study. It comprised 2,532 postmenopausal women with osteoporosis receiving PTH (1-84) or placebo. The follow up was up to 18 months. The main goal was the reduction in vertebral fracture

risk. Mean age of patients in the study was 64 years, and of them, 19% had at least one vertebral fracture. After 18 months, the increase in BMD at the lumbar spine in women treated with PTH (1-84) was 7% compared with the placebo group. The risk of new vertebral fractures decreased by 66% in the group treated with PTH. Hypercalcemia was observed in 28.3% of treated women, compared to 4.7% in the control group. (Greenspan et al., 2007)

3.4.2 Combination and sequential therapy

The effects of concurrent or sequential therapy with PTH and antiresorptive agents have been studied. Black et al. compared the effects of PTH (1-84), alendronate, or both in combination in postmenopausal women in the study PaTH. In this study, 238 postmenopausal women were randomized with ages comprised 55-85 years with a T-score <-2.5 or a T-score <- 2 and with at least one risk factor or additional fracture. Initially there were 3 groups receiving: PTH (1-84) 100 mcg/day + placebo (n=119), alendronate (ALN) 10mg/day + placebo (n=60) and PTH (1-84) 100 mcg/day + ALN 10 mg / day (n=59). All participants received daily calcium 500mg + vitamin D (400UI) supplements. (Black et al., 2003)

At one year, spine DXA had increased in all three groups. There was no difference in spine DXA between the PTH group and the combination group. However, the PTH group had a significantly greater increase in volumetric BMD of the spine on quantitative CT than the alendronate and combination groups. Volumetric trabecular lumbar BMD increased with respect to baseline by 26%, 13% and 11% in the PTH alone, PTH and alendronate and alendronate group respectively at 12 months. Similarly, volumetric trabecular BMD of the total hip increased by 9%, 6% and 2% respectively in the 3 groups.

3.4.3 Effects on bone architecture

In spite of the facts of all these studies previously published, data about what was happening in bone was lacking. Recker et al. studied bone biopsies from iliac crest from postmenopausal osteoporotic women who received placebo (n=8) or 100 mcg PTH(1-84) for 18 (n=8) or 24 (n=7) months to assess cancellous and cortical bone formation and structure. Using micro CT and histomorphometry at 18 months, cancellous bone volume (BV/TV) measured was 45-48% higher in subjects treated with PTH(1-84) versus placebo, and also resulted in a higher trabecular number (Tb.N) and thickness. The higher Tb.N appeared to result from intra-trabecular tunneling. Connectivity density was higher and structure model index was lower, indicating a better connected and more plate-like trabecular architecture. Cancellous bone formation rate (BFR) was 2-fold higher in PTH (1-84)-treated subjects, primarily because of greater mineralizing surface. (Recker et al.,2009)

3.4.4 Adverse effects

The physical effects produced by PTH 1-84 are in most cases mild. The most common is hypercalcemia, present in 28% of women treated vs. 4.6% in the placebo group and hypercalciuria, 46% versus 23% respectively. However, the number of withdrawals of treatment due to this cause was rare in published clinical trials (two patients in the PaTH study and six patients in the TOP study) and generally the effect was controlled by removing the supplements of calcium and vitamin D. Although it is believed that hypercalcemia could slightly modify electrocardiographic studies decreasing the QT interval without significant changes or minimal changes in heart rate, PR interval or QRS duration and axis, no differences between groups were observed. Other reported side

effects, although often not as important as the ones mentioned above, were nausea and vomiting. Fisher et al, reported a study with 344 rats treated with nearly life-long daily teriparatide, and found an increased risk of osteosarcoma. Nevertheless, to date there is no reported increase in prevalence of osteosarcoma in humans treated with neither teriparatide nor PTH 1-84, and no association has been found between primary hyperparathyroidism and osteosarcoma. Recently, Tashjian et al. reported that they had not collected a single case of osteosarcoma in humans, following the prescription of more than 250,000 treatments with PTH, 1-34 and 1-84 after follow-up of patients who participated in the studies with PTH 1-84 in the 80's. (Tashjian & Goltzman, 2008)

4. Dual action agents

4.1 Strontium ranelate

Strontium (Sr) is a chemical element with an atomic number 38. It is an alkaline earth metal and was isolated for the first time, as an impure substance, in 1808 in a Scottish city named "Strontain" from which this element received its name. It is in this city where strontium is found in higher concentrations than usual (73g/kg). The earth cortex contains 0.042% of strontium and is as abundant as chloride and sulfur. It can also be found in rocks, dust, carbon and oil, as well as in some foods as cereals, green vegetables and milk. In marine water, strontium is the most abundant trace element, reaching values of 8 mg/L. (Cabrera et al., 1999) In its natural state, called stable strontium, this element is not radioactive and it is harmless. The only compound harmful for the human being is strontium chromate, and due to chrome not to strontium. (Levy et al., 1986) The therapeutic potential of strontium was discovered around 1940, when strontium-89 was used as an analgesic agent in bone metastases from prostate cancer. (Giammarile et al., 1999; Saarto et al., 2002) Afterwards this isotope, together with strontium-88, have been used as markers for calcium absorption. (Cabrera et al., 1999)

4.1.1 Pharmacological characteristics

The main entrance of strontium into the body is through the gastrointestinal system. The skin and the lungs can also absorb it. Its gastrointestinal absorption varies with age and has a very high variability in infants. In the elderly, the fluctuation is about 10%. A number of absorption mechanisms have been proposed, beginning with the passive mechanisms and diffusion, to transporter mediated absorption, as proposed by Papworth et al. Strontium absorption augments with fasting and it is seriously affected by calcium, phosphorus and other chelating agents in the bowel and its absorption rate is about half of calcium. Other experimental studies have demonstrated that during pregnancy and breast-feeding strontium absorption is increased, reaching a maximum during breast-feeding. (Papworth & Patrick 1970; Papworth & Vennart, 1984)

Absorption of strontium is dose dependant. Its bioavailability decreases with a lower dose, confirming that, just like calcium, absorption involves passive diffusion, independent of vitamin D levels, as well as saturable active transport, regulated by vitamin D and a facilitated diffusion. (Ardissino et al., 2000) Studies in a variety of animals (i.e. rats or monkeys) demonstrate that the pharmacokinetic data of renelic acid have a high variability. It is estimated that its oral absorption is poor and slow, probably due to a deficient intestinal permeability. (Li et al., 2006)

Serum concentration of strontium can be affected by the administration together with calcium and with meals. When administered together with 0,5 grams of calcium, strontium relative bioavailability decreased 57%, 63% when administered with meals and 71% when administered with calcium and meals. Due to these absorption difficulties, several studies were conducted in order to determine the best mode of administration. Comparing strontium administration one hour before breakfast and three hours after breakfast to every 12 hours resulted in a decrease of bioavailability of 46 and 55% respectively. In phase 2 studies no difference was observed between giving strontium 1 gram every 12 hours or 2 grams before bedtime. Thus, to guarantee the best absorption and bioavailability, it is recommended to administer strontium ranelate two hours after dinner. (Leeuwenkamp et al., 1990; Reginster & Meunier 2003) Vitamin D seems to increase the medications' absorption, though in phase 3 studies it was observed that the influence of vitamin D did not change strontium availability in more than 10%, which is clinically insignificant. (Ardissino et al., 2000; Leeuwenkamp et al., 1990; Marie, 2003)

Strontium excretion is mainly through renal clearance, and to a lesser extent through feces and sweat. In healthy adults, strontium plasma clearance varies between 9.4 and 11.7 ml/min, meanwhile the urinary clearance is between 4.0 and 5.4 ml/min. (Papworth & Vennart, 1984; Leeuwenkamp et al., 1989) In animal studies, the bone tissue strontium content decreased to approximately 50% at week 6 to 10 after stopping treatment. Renelic acid, given its high polarity, is poorly absorbed and its half-life in animals is about 1 hour, though it varies according to its absorption. In humans, renelic acid excretion is approximately 78 ml/min and therefore it has a half-life of 2.6 hours. (Li et al., 2006; Leeuwenkamp et al., 1990)

Due to its chemical properties, strontium can form complexes with oral tetracyclines and quinolones, and therefore its administration with these medications is not recommended. Strontium administration together with diuretics could increase its serum concentration around 20%. This effect is greater with thiazide diuretics, furosemide and indapamide, and could be explained by the increase in the strontium tubular re-absorption, together with calcium, which would rise in parallel. Even though this increase in strontium levels is not clinically significant and no dose adjustment is needed. Magnesium and aluminum hydroxide can significantly decrease strontium bioavailability, therefore it is not recommended to take these medications at night, when strontium should be administered. (Leeuwenkamp et al., 1990; Marie, 2003)

4.1.2 Mechanism of action

In-vitro, strontium ranelate increases collagen and non-collagen protein synthesis thru mature osteoblasts. The bone forming effects were confirmed with the increase in the replication of pre-osteoblastic cells. This stimulus of the replication of the pre-osteoblastic cells and the increase of collagen and non-collagen proteins are the reason why strontium ranelate is considered as a dual effect bone agent, since it does not only decrease resorption. (Bonnelye et al., 2008) In an in-vitro assay of isolated rat osteoclasts, the pre-incubation of bone slices pre-treated with strontium ranelate, demonstrated a dose dependant decrease in bone resorption activity. In another assay, using chicken bone marrow, a dose dependant decrease in the expression of carbonic anhydrase II and the alpha subunit of the vitronectin receptor could be observed. (Takahashi et al., 2003; Caverzasio, 2008; Bonnelye et al., 2008; Reginster et al., 2003)

The main mechanism of action that rules bone resorption at a molecular level is the RANK/RNAKL/OPG system described earlier. Concentrations of 0.1 mM to 2 nM of strontium ranelate, decrease the ability of human osteoblasts of inducing osteoclast differentiation, by decreasing expression of mRNA of RANK-L and increasing mRNA expression of OPG, according to the studies done by Brennan et al in 2006. (Close et al., 2006; Chapurlat & Delmas, 2004)
The human body has a very strict extracellular calcium control mechanism. This control is performed by varied body tissues with the aim of keeping extracellular calcium levels within a narrow range, which is essential for the normal cellular function including muscular contraction, nerve impulse transmission or platelet aggregation, for example. The tissues involved in this task, like the chief cells of parathyroid glands, C cells of the thyroid glands, renal tubules, the cortical ascending limb of the nephron, the intestinal epithelium and the bone cells like osteoclasts and osteoblasts, express a receptor capable of detecting changes in the extracellular calcium levels and act according to the requirements, these receptors are called calcium receptors or calcium sensing receptors (CaR). (Brown 2003)
Several studies with cell cultures have been able to demonstrate that this receptor can be activated by other divalent cations, including strontium, which just as calcium but with a lower potency can activate the CaR. This means that in the presence of strontium, chief cells of the parathyroid glands will decrease its secretion and that osteoclasts will decrease bone resorption, for example. Numerous assays demonstrate that 0.13 nM strontium plasma levels, like the ones seen in patients treated with 2 g of strontium ranelate daily, won't affect the CaR in soft tissues. The effect on osteoclast apoptosis could be related to the activation of the transmembrane receptor attached to phopholipase C (CaR) and mediated by an independent signaling pathway of IP3-protein kinase C. Some other authors suggest that besides CaR, there are other receptors that can also influence these actions. These receptors could be related to the stimulating effect of strontium on osteoblasts replication in CaR knockout rats. (Arlot et al., 2008; Brown, 2003)

4.1.3 Effects on other tissues

Some studies have demonstrated that strontium ranelate has beneficial effects in tissues other than bone. Taking cartilage for example, strontium ranelate increased basal production of proteoglycans stimulated by insulin growth factor 1 by chondrocytes of young subjects, old subjects and subjects with osteoarthritis. On the contrary, it showed no effect on the proteoglycans production induced by interleukin 1 (IL-1), the stromelycin production stimulated by IL-1β or chondrocytes activity. These findings suggest that strontium ranelate stimulates human cartilage matrix formation in vitro without activating the chondroresorption process. (Henrotin et al., 2001)

4.1.4 Effect of strontium ranelate in fracture reduction

It is known that due to the chemical properties of the compound (a higher molecular weight), densitometric values of patients treated with strontium ranelate will be higher than the true values. There are many studies that have measured the influence of the chemical characteristics of strontium on densitometry and have developed some mathematical formulas to remove this influence from the DMO value. These formulas are a little bit complicated and require too much time to put them into practice in the daily practice,

therefore the utility of these formulas is restricted to almost only research. It is easier and almost accurate to calculate that half of the DMO gained in the first year of treatment with strontium ranelate is due to an increase on BMD and the rest is due to the higher molecular weight on strontium measured by the DXA. (Blake & Fogelman, 2006b)

Currently we have data from clinical studies comparing strontium ranelate to placebo for fracture prevention for up to five years. Moreover we have data of fracture incidence in patients treated with strontium ranelate for up to 10 years. The phase III pivotal trial was performed in 75 centers distributed through 12 countries worldwide. It was structured in three different clinical trials. The FIRST (Fracture International run-in for Strontium Ranelate) trial mean duration was 101 days (SD ± 52) and was performed with the objective of normalizing calcium and vitamin D levels of all subjects. From this trial the other two were derived, the SOTI (Spinal Osteoporosis Therapeutic Intervention) trial and the TROPOS (Treatment of Peripheral Osteoporosis) trial. (Reginster & Meunier, 2003; Reginster et al., 2005) The main propose of these trials was to evaluate the effect of strontium ranelate in axial and appendicular skeleton as well as the tolerability in postmenopausal osteoporotic women. Their main objective was to calculate the reduction in the incidence of new vertebral fractures (SOTI trial) as well as non-vertebral fractures (TROPOS trial). (Reginster et al., 2008) The analysis included 1649 postmenopausal women in the SOTI trial and 5091 patients in the TROPOS trial. In the SOTI trial women were randomized into two groups, a placebo (control) group and another one receiving 2 g daily of strontium ranelate for a period of four years. In the fifth year, the patients taking placebo switched to strontium ranelate and 50% of the ones taking strontium ranelate switched to placebo. In the TROPOS trial, all patients remained in their original treatment group during the whole 5 years. The preliminary three-year results showed a vertebral fracture reduction of 41% with a NNT of 9. Furthermore an increase in BMD of 12.7% was observed. The vertebral fracture reduction at the end of the forth and fifth year was of 33% and 24%, respectively. Similar results were obtained from the TROPOS trial where the vertebral reduction rate was 39% at the end of the third year and 24% at the end of the fifth year. Regarding the non-vertebral fractures the decrease in the relative risk of fracture with strontium ranelate was 16% at the end of the third year and 15% at the end of the fifth. A post-hoc analysis of these data in a subgroup of 1,977 patients with high fracture risk (≥74 years old and a T-score of ≤-2.4) showed a vertebral fracture risk reduction of 36% at the end of the third year and 43% at the end of the fifth. (Blake & Fogelman, 2005; Reginster et al., 2007; Moro-Alvarez & Diaz-Curiel, 2007)

Since there is no data showing the fracture reduction risk of strontium ranelate in placebo controlled patients, Reginster et al, compared the fracture incidence between the original strontium ranelate group at the end of the fifth year to the strontium ranelate group followed for ten years. The results are shown in table 2. (Reginster et al., 2010) These results show no statistically significant differences between the incidence of fractures at the end of year 5 or 10. An important fact to bear in mind is that the sample was significantly reduced since at the end of the tenth year, just 233 patients continued in the follow up study.

Another sub study performed by Seeman et al, with patients over 80 years of age showed a reduction in the vertebral fracture risk of 32% in the third year and 31% in the fifth year. For peripheral fractures, the reduction in fracture risk was of 31% in the third year and 27% in the fifth year, and for hip fractures the risk reduction was 32% in the third year and 24% in the fifth. (Seeman et al., 2010; Seeman et al., 2006)

	5 years with placebo	5 years with Strontium ranelate	10 years with strontium ranelate
Vertebral fracture incidence	24.9%	18.5%	20.6
Non-vertebral fracture incidence	20.9%	12.9%	13.7%

Table 2. Fracture incidence at 10 years. (Reginster et al., 2010)

4.1.5 Adverse events

At the end of the third year, in the phase III studies, the only adverse event that showed statistically significant differences compared to placebo was diarrhea, found in 6% of the patients taking the drug and 3.6% of the placebo group. Other adverse events found to be more frequent with strontium ranelate, but with no statistically significant difference compared to placebo, were nausea, headache, dermatitis, eczema, and thrombo-embolic events. The latter was studied thoroughly, but no relation to the drug was found. Other studies with high doses of strontium ranelate have been performed in order to investigate thrombo-embolism, but no alteration in coagulation parameters have been found to support the finding in the phase III trial. (Blake & Fogelman, 2006a; Halil et al., 2007; Liu et al., 2009; Ulger et al., 2010)

At the end of the fifth year some other events were found to be more frequent (with no statistically significant differences compared to placebo) such as memory loss, cognitive impairment and seizures. The rest of the adverse events had the same incidence in the study drug and the placebo group. (Blake & Fogelman, 2006a; Liu et al., 2009)

Strontium ranelate has been used widely in Europe and there is a rare adverse event reported to be due to treatment with this compound. Reports state that 0% to 8% of patients suffer a drug rash with eosinophilia and systemic symptoms (DRESS), which is an allergic reaction to the medication that usually appears between 3 and 6 weeks after starting treatment. This syndrome can be fatal if the medication is not stopped and treatment with glucocorticoids started. (Musette et al., 2010)

5. Future therapies

5.1 Cathepsine K (CatK) inhibitors

Human cathepsin K is a 329 amino acid long protein consisting of an N-terminal 15 amino acid long signal sequence, a 99 amino acid long propeptide, and a 215 amino acid long catalytic unit. It shares about 60% protein sequence identity with cathepsins L, S, V and less than 35% with cathepsins F, O, B, H, and W. Cathepsin K is expressed predominantly in osteoclasts and various other multinucleated cells such as giant foreign body cells and Langhans cells. To a lesser degree it is found in macrophages, synovial fibroblasts, and fibroblasts at locations of wound healing or inflammation, chondrocytes, various epithelial cells of the human fetus, adult lung airway epithelium, thyroid epithelium, and possibly at low concentrations in smooth muscle cells. Once the enzyme is synthesized, it is sequestered into lysosomes and can be secreted into the extracellular environment. It is specifically secreted into the resorption lacuna underneath actively resorbing osteclasts where it is responsible for the degradation of the collagen type I dominated organic bone matrix. Thus, similarly to pycnoidisostosis, elimination of cathepsin K in osteoclasts results in inhibition of bone resorption. Inhibitors of cathepsin K are suggested to have less of an effect on

osteoclast–osteoblast interaction, resulting in less inhibition of bone formation, than available bisphosphonate antiresorptive agents. Human cathepsin K inhibitors have been shown to prevent bone loss in ovariectomized mice without blunting the anabolic action of parathyroid hormone (PTH).
Although no CatK inhibitor is currently marketed for osteoporosis treatment or prevention, studies of three CatK inhibitors for the treatment of osteoporosis have been reported: balicatib, relacatib, and odanacatib.
The most commonly used drugs for the treatment of osteoporosis inhibit osteoclast-mediated bone resorption. Osteoclasts are hematopoietically derived multinucleated giant cells that resorb bone by focal attachment and demineralization, followed by the enzymatic degradation of organic bone matrix. The demineralization is achieved by the secretion of acid onto the bone surface. The organic matrix (mainly type 1 collagen, the principal bone matrix protein) is degraded primarily by the enzymatic action of cysteine proteases, particularly cathepsin K (CatK). CatK is the most abundantly expressed cysteine protease in osteoclasts and exhibits collagenolytic activity under acidic conditions. Currently treatment of osteoporosis, like bisphosphonates prevent acid secretion by disruption of the ruffled border and proton pump required for hydrogen ion secretion.
The collagenases of the matrix metalloproteinase family have been considered as the main proteases for the degradation of collagen as they were thought to be the main ones capable of cleaving triple helical collagen. However, matrix metalloproteinase are active at neutral to slightly alkaline pH values whereas at the site of bone resorption, within the resorption lacuna, acidic pH conditions prevail. Thus, acidic lysosomal hydrolases were proposed to operate as the main collagen degrading proteases. Previously, only Cathepsins B and L were known. Cathepsins B and L were thought to be the key factors, as both enzymes were known to cleave in the telopeptide region of triple helical collagens. However in the early 1990's a new cathepsine was identified thanks to DNA clonation techniques. Initially this new cathepsine was identified only in osteoclasts and was called cathepsine O, later its name changed to cathepsine K. This protease exhibited a potent collagenase activity towards the main connective tissue collagens type I and II, and immunohistochemical analyses revealed a predominant but not exclusive expression in osteoclasts. After that, pycnodysostosis, a hereditary form of osteopretosis was related to a low level of Cathepsine K due to a complete deficiency.

5.1.1 Balicatib

Balicatib is highly selective for CatK in enzyme assays but has lesser selectivity in living cells. In vitro studies have shown that a basic moiety in its chemical structure results in its accumulation in the acidic environment of the lysosomes at concentrations sufficient to inhibit cathepsins B and L and possibly others. Clinical studies of balicatib have demonstrated BMD increases in postmenopausal women, but treatment was associated with cutaneous adverse events. The first demonstration of the effect of cathepsin K inhibitors on bone density in humans was seen with balicatib. This trial, published by Adami et al., in an ASBMR meeting in 2009 (Denver, CO, USA) was a multicenter, randomized, placebo-controlled, 12-month, dose-range finding study of 675 postmenopausal women with lumbar spine T-score less than 2.0. In the group that received 50mg of balicatib daily, markers of bone resorption declined by more than 55% with no decline in markers of bone formation (osteocalcin, bone-specific alkaline phosphatase and N-terminal propeptide of type I

collagen). The BMD in the lumbar spine increased 4.46% and 2.25% in the total hip. Skin reactions, including pruritus and morphea-like changes, were noted in a small number of patients. In a small Japanese trial, intact PTH levels were shown to increase by 50% with balicatib treatment.

5.1.2 Relacatib

Relacatib is a potent but nonselective inhibitor of cathepsins K, L, V, and S for which no clinical information has been published. Administration of relacatib to ovariectomized and control monkeys resulted in an acute and rapid reduction of bone markers, and this effect lasted for up to 2 days depending on the dose delivered.
Due to side effects, especially skin reactions, drug development of all cathepsin K inhibitors has been suspended or slowed down except for odanacatib and currently ONO 5334.

5.1.3 Odanacatib

Odanacatib is a powerful, reversible nonpeptidic biaryl inhibitor of cathepsin K that inactivates the proteolytic activity of cathepsin k. It is synthesized by replacing the P2-P3 amide bond of an aminoacetronintrile dipeptide 1 with a phenyl ring. This results in a powerful, selective inhibitor with the capacity to inhibit cathepsin K in osteoclasts. (Bromme & Lecaille, 2009)
Two studies have been carried out to evaluate the efficacy and safety of odanacatib, a phase I study to determine the dose and a phase II study to evaluate the safety and efficacy. In the Phase I study a group of 49 women was used to evaluate a weekly dose. Doses of 5mg, 25 mg, 50mg, and 100 mg were used and 12 women were assigned to the placebo group. A group of 30 women was used to evaluate the daily dose. Doses of 0.5, 2.5, and 10mg were used, with 6 women assigned to the placebo group. All doses were administered in fasting conditions. Odanacatib had a long half-life of between 66 and 93 hours for all the regimes and doses used. The efficacy of weekly, and daily doses in modifying the markers was evaluated. The effect was dose-dependant although not dose proportional. Reductions in resorption markers were greatest for doses >50 mg weekly and doses ≥2.5mg daily. Maximum suppression was achieved between day 3 and day 5 with the weekly dose and was maintained until the following dose. (Stoch et al., 2009)
The Phase II trial published by Cusick et al in the ASBMR meeting in 2009 (Denver, Co, USA), was a double-blind, randomized, placebo-controlled trial of 12 months duration with an anticipated extension period of 24 months. It included 399 post-menopausal women (postmenopausal (5yr) or bilateral oophorectomy) between 45 and 85 years,with a T-score <-2 but not less than -3.5 at any site. Patients were divided into five groups according to the dose: placebo, 3 mg/weekly, 10 mg/weekly, 25mg/weekly and 50 mg/weekly. The changes in BMD at the lumbar spine were assessed and considered a primary objective. Also changes in bone remodeling, changes in BMD in other sites and adverse effects were evaluated. The results showed a dose-dependant increase in BMD in all sites. The greatest increase was obtained with the highest dose. Weekly administration of 50mg of odanacatib increased bone mass by 5.7% in the lumbar spine, 4.1% in the total hip, 4.7% in the femoral neck, 5.2% in the trochanter and 2.9% in the distal third of the radius at 24 months. Resorption markers fell in a dose-dependant manner from the beginning of treatment and remained reduced during the first six months, after which they increased and the differences with placebo disappeared.

The results of the extension of the phase II study to 36 months (published by Eisman et al at the ASBMR meeting 2009 in Denver, included 169 women who were randomized to odanacatib 50 mg and placebo weekly. In the odanacatib group, BMD continued to increase (lumbar spine 7.5%, total hip 5.5%, femoral neck 5.5% and trochanter 7.4%). The urine NTX resorption marker was 50% lower compared with placebo, whereas there were no differences in the BSAP (bone specific alkaline phosphatase) formation marker. At three years, formation markers were not only not reduced, but in fact increased by 18% over baseline values.

5.1.4 ONO 5334

ONO-5334 is a new cathepsin K inhibitor. There has been a first study to investigate the efficacy and safety of ONO-5334 in postmenopausal osteoporosis. This was a 12-month, randomized, double blind, placebo and active-controlled parallel-group study conducted in 13 centers in 6 European countries. Investigators included 285 postmenopausal women aged 55 to 75 years with osteoporosis. Subjects were randomized into one of five treatment groups: placebo; 50 mg twice daily, 100 mg once daily, or 300 mg once daily of ONO-5334; or alendronate 70mg once weekly. After a year of follow up all ONO-5334 doses and alendronate showed a significant increase in BMD at the lumbar spine, total hip (except 100 mg once daily), and femoral neck. There was little or no suppression of ONO-5334 on bone-formation markers compared with alendronate, although the suppressive effects on bone-resorption markers were similar. There were no clinically relevant safety concerns. With a significant increase in BMD, ONO-5334 also demonstrated a new mode of action as a potential agent for treating osteoporosis. This new drug increases the armamentarium not only in cathepsin K inhibitors (the second that seems to be available) but also in osteoporosis treatment. (Eastell et al., 2011)

In conclusion, Cathepsine K inhibitors are a new family of drugs that increase the armamentarium in the fight against fractures, as the most dangerous effect of osteoporosis. Having the possibility to treat this disease in different points of the resorption pathway is positive and it gives us the possibility to reach a better and easier way to decrease the incidence of fractures.

5.2 Anti-sclerostin monoclonal antibody

Sclerostosis is a rare autosomal-recessive disorder. Patients with this disease characterize for having a high bone mass. (Hamersma et al., 2003; Beighton, 1988; Barnard et al., 1980) The study of its etiopatogenesis led to the discovery of sclerostin, a protein that in humans is encoded by the SOST gene. (Balemans et al. 2001; Brunkow et al., 2001) It is classified as a key inhibitor of osteoblast-mediated bone formation. (Poole et al., 2005; Wergedal et al., 2003) Loss-of-function mutations in this gene are associated with sclerosteosis, which causes progressive bone overgrowth and increases in bone mass and BMD.

Another similar disease is van Buchem disease, which is a milder form of sclerostosis and is caused by a deletion downstream of this gene, with a consequent reduced sclerostin expression. SOST gene knock out mice don't produce sclerostin and have a high bone mass, confirming the effect of this protein on bone mass and BMD. Besides the increase in bone mass and BMD taking place from sclerostin deficiency, there have been no reports of fractures in individuals with sclerosteosis or van Buchem disease. (Hamersma et al., 2003; Wergedal et al., 2003)

Sclerostin binds to low-density lipoprotein receptor-related protein (LRP) 5/6 and blocks Wnt-signaling, negatively regulating bone formation and in that way, inhibiting osteoblast differentiation, proliferation, and activity. (Baron & Rawadi 2007) Thus, the inhibition of sclerostin was thought to have therapeutic potential in treating human bone metabolism defects such as systemic bone loss, focal bone loss, fracture healing, and orthopedic procedures where increases in bone formation, bone mass, bone mineral density (BMD), and consequently bone strength, are sought-after. (Ott, 2005)

5.2.1 Sclerostin inhibition

Rats treated with a sclerostin antibody experience a reversal of estrogen deficiency induced bone loss at several skeletal sites. Additionally, an increase in bone mass and strength is observed in treated rats compared with controls. Similar results were observed in treated monkeys. In models of fracture healing in mice and rats, treatment with a sclerostin antibody increased bridging and bone strength at sites of fracture, resulting in enhanced bone healing compared with controls. (Padhi et al., 2011)

AMG 785 is a high affinity immunoglobulin G2 (IgG2) monoclonal antibody generated by humanizing a mouse sclerostin monoclonal antibody that neutralizes sclerostin. The first-in-human single-dose study in healthy men and postmenopausal women was performed to evaluate pharmacokinetics, pharmacodynamics, tolerability and safety of doses of 0.1, 0.3, 1, 3, 5 or 10 mg/kg sub-cutaneus and 1 or 10 mg/kg intravenous of AMG 785. A total of 72 subjects participated in the study and were followed for up to 85 days. Study product pharmacokinetics was nonlinear with dose. Dose-related increases in bone formation markers and decreases in bone resorption markers were observed. A small percentage of the patients developed anti-investigational product bodies but most of them were non-neutralizing antibodies. The medication was well tolerated. (Padhi et al., 2011) A phase II study of 419 postmenopausal women with low BMD has been started to compare the efficacy of sclerostin neutralization with alendronate and teriparatide. (Rachner et al., 2011) Finally, a multicenter, phase IIa, randomized double-blind, placebo controlled, multi-dose study is ongoing to evaluate safety, tolerability, pharmacokinetics, and phamacodynamics of AMG 785 in postmenopausal women with low bone mass. In conclusion, anti sclerostin antibody treatment could be the most effective treatment for osteoporosis and bone defect related diseases. Even though, we will have to wait until all the ongoing and planned trials are over to analyze the data and have access to this kind of treatment.

6. Conclusion

During the last 10 years, new therapeutic agents have emerged among the pharmacological treatment options for osteoporosis. The newer options belong to new families with optimized mechanisms of action, allowing us to restore the lost bone mass quicker and more effectively than with the old medications. Nevertheless, one has to be conscious that all treatment options have specific indications and a wide range of adverse events, that have to be taken into consideration before making any decision. Moreover, it has to be remembered that most treatments for osteoporosis have to be given concomitantly with changes in lifestyle and/or calcium and vitamin D supplementation. New therapies are in development that probably will allow us to treat for a shorter time obtaining better results for our patients.

7. References

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The Role of Hormone Replacement Therapy (HRT) and Tibolone in the Prevention and Treatment of Postmenopausal Osteoporosis

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1. Introduction

Life expectancy has increased considerably in recent decades thanks to the improvement of measures for health protection and disease prevention as well as improvements in the quality of health systems. While in the middle of last century the average life expectancy of a woman was about 50 years, today there are over 350 million women older than 60. Furthermore, from the perspective of developed countries, postmenopause is a stage that spans more than one third of the life of a woman. That is why the research on the pathophysiology and treatment of menopause has become necessary and important.

The decrease in sex steroid production by the ovary that occurs in perimenopause and menopause is associated with a rapid loss of bone mass due to increased resorption. In the past two decades, multiple observational studies have noted the beneficial effect of hormone replacement therapy (HRT) in postmenopausal women's health, based mainly on the relief of symptoms associated with estrogen deprivation such as vasomotor and genitourinary symptoms. These studies also indicated a preventive effect on aging-related diseases such as osteoporosis. Estrogens have been shown to be effective in increasing bone mineral density (BMD) and prevent fractures, but information on side effects from long use has reduced their use for the treatment of osteoporosis (Tamborini & Ruiz, 2004).

Tibolone is a synthetic steroid used in the treatment of postmenopausal symptoms and decreased libido that has an estrogen agonist effect on bone.

Although the intimate mechanisms of control of bone remodelling are not still completely known, we do have enough information to say that estrogens have a role in the homeostasis of the skeleton, which is why their decline is associated with reduced bone mass, impaired in the microarchitecture and increased risk of fracture.

The following chapter discusses the role of estrogen therapy and tibolone in the prevention and treatment of postmenopausal osteoporosis.

2. Literature search

Studies with English language abstracts identified in MEDLINE, HealthSTAR, and Cochrane Library databases from 1990 to 2010 are reviewed. Reference lists of key articles

and meta-analyses have also been reviewed. We used all published studies of HRT and tibolone if they contained a comparison group of HRT nonusers and reported data relating to HRT use and clinical outcomes of interest. Studies have been excluded if the population was selected according to prior events or presence of conditions associated with higher risks for targeted outcomes.

3. Hormone replacement therapy (HRT)

3.1 Effect of sex steroids on bone

The possible adverse effects of estrogen deficiency on the bone metabolism are known since the 40 decade (Albright, 1940, 1947). The first studies confirmed a higher rate of oophorectomies among osteoporotic women than in general population, and that the surgical treatment had place earlier than the age for natural menopause. These studies also showed that the negative balance of osteoporosis was normalized with the administration of estrogen. Thus it was postulated that estrogen somehow stimulated the action of osteoblasts, which is now accepted as one of the potential mechanisms of action of estrogen on bone mass (Lindsay, 1995).

Oophorectomized and postmenopausal women have decreased circulating levels of other steroids in addition to estrogens (Lindsay, 1995). In women, circulating androgen concentrations are in the order of nanomoles or micromoles while estrogens do so at concentrations on the order of picomoles. The concept of androgen deficiency syndrome is relatively old, but in recent years there has been a renewed interest in the subject.

The premenopausal ovary produces significant amounts of progesterone during the luteal phase of each cycle. Progesterone appears to act directly on bone turnover and may play a role in the relationship between bone resorption and new bone formation (Prior, 1990).

Although we do not yet fully understand the bone turnover process or control, there is sufficient information to conclude that sex steroids play an important role in skeletal homeostasis. The lack of secretion of ovarian sex steroids results in a net loss of bone tissue. When given to women with deficiency of sex steroids, these hormones reverse many of the effects related to loss of ovarian function. Therefore, it has been suggested that postmenopausal women should take HRT long term to prevent these negative effects, including osteoporosis and fractures.

3.1.1 Estrogens and bone

With the decrease in estrogen levels that occurs at menopause, there is an increase of bone remodelling with a loss of balance between formation/resorption, predominantly the latter. HRT decreases the elevated levels of resorption to those before menopause. Bone cells have estrogen receptors (Vidal et al., 1999). The most important action of estrogen on bone is to inhibit bone resorption. This action indirectly regulates the production of cytokines and growth factors in osteoblasts. Since there are estrogen receptors in osteoclasts, may also be logical to think that there is a direct action. Inhibition of bone resorption by estrogen is probably the conclusion of inducing apoptosis in osteoclasts (Kameda et al., 1997), this action being probably due to increased TGF-β. Estrogens have shown to increase the proliferation of osteoblasts and the expression of different genes that encode enzymes, bone matrix proteins, transcription factors, hormone receptors, growth factors and cytokines. However, these results have varied depending on crop models (Manolagas, 2000). Estrogens

have also shown the ability to inhibit TRAP expression or inhibit certain steps in the RANK-JNK signal (Srivastava et al., 2001).

The current idea of the action of estrogen is that it is through different pathways. There is, first, an antiapoptotic effect of estradiol on osteoblasts and osteocytes due to rapid non-genomic action (Kousteni et al., 2001). It has succeeded in synthesizing a ligand called ESTREN that act exclusively through this channel, and theoretically could have the same effect as estrogens on bone without the genomic consequences of these. This model has been named to a new class of pharmacological agents called ANGELS (Activators of NonGenotropics Estrogens Like Signalins), and there would be another apoptotic action on osteoclasts (Manolagas et al., 1995). The actions of estrogen on bone cells, are inhibition of bone resorption by decreasing the synthesis or response to interleukins such as IL-6, IL-1 and TNF-α with less differentiation of precursor cells into osteoclasts by increasing IL-4. Among other effects, estrogens decrease lytic enzyme activity of osteoclasts and produce changes in growth factors insulin-like IGF-I, IGF-II and interferon types α, β and γ. The effect of estrogen is mediated in part by growth factors and interleukins such as IL-6, which is a potent stimulator of bone resorption by blocking estrogen synthesis by osteoblasts. Estrogen may also antagonize the interleukin receptors. The apoptosis of osteoclasts is also regulated by estrogens. Faced with reduced levels of estrogen, osteoclasts live longer and have greater capacity of absorption. Estrogens regulate tumor growth factor system associated with the RANK/RANK-L resulting in a decrease in the activity of osteoclasts. They also stimulate the production of osteoprotegerin (OPG) by osteoblasts. Thus, the presence of estrogen prevents binding of RANK-L to RANK resulting in inhibition of the formation, differentiation and survival of osteoclasts (Eghbali-Fatourechi et al., 2003). In response to increased bone resorption, it exists an increase in bone formation, creating a high turnover that leads to bone loss and deterioration in the microarchitecture. In the first 5 years since menopause, a substantial disruption of the trabecular architecture can be observed, demonstrated by the analysis of iliac crest biopsies using computed microtomography techniques (Issever et al., 2002). Once broken the continuity of a trabecula, we can increase thickness, but will not get a new connectivity.

We could conclude that steroid hormones are involved with a complex action system clearly influencing the bone marrow regulation. They are part of the mechanism regulating RANK-RANKL-osteoprotegerin, whose predominant action is bone resorption, and it is performed through genomic and nongenomic actions. In addition, estrogens also act through indirect mechanisms of action such as reducing the sensitivity of the bone to resorptive effects of parathyroid hormone (PTH), acting as antiresorptive agents. They produce an initial decrease in serum calcium and, therefore, a transient increase in PTH and calcitonin secondary modifications. Due to the increase in α 1-hydroxylase activity and phosphorus decreased, they produce an increase in hydroxyvitamin D3. Estrogens also increase the intestinal absorption and decrease renal excretion of calcium, and have direct effects on the secretion of PTH and calcitonin.

3.1.2 Androgens and bone

Androgens have a profound effect on bone and muscle physiology in women. Both for their intrinsic activity as for their conversion to estrogens, androgens have a modulatory effect on bone remodelling cycle. The androgen deficiency, like estrogen, may facilitate the development of osteoporosis. Androgenic anabolic steroids are sometimes used in the

treatment of osteoporosis but their use is limited by side effects of virilizing type. Evidence of the effects of androgens on bone mass comes from women with polycystic ovary syndrome or steroid-secreting ovarian tumors in which there is an increase in bone mineral density (Gregoriou et al., 2000). On the other hand, we know that the combination of androgens and estrogens for hormone replacement therapy in menopausal women is associated with increased bone mass above that observed with estrogen alone (Castelo-Branco et al., 2000). A cohort study developed to assess BMD in postmenopausal women using estradiol and testosterone hormonal implants comparing to that of patients without hormonal therapy, confirmed that BMD variance between the groups in the period of 1 year was significantly different, and concluded that the combination of estradiol and testosterone promoted bone protection in postmenopausal women (Britto et al., 2011).

Androgen receptors have been identified in osteoblasts, osteoclasts and osteocytes. Androgens stimulate the proliferation and differentiation of osteoblasts, stimulate the synthesis of extracellular matrix proteins, and stimulate mineralization. These steroids affect the functionality of bone cells through their effects on local factors that control bone cell microenvironment, have proapoptotic effects on osteoblasts and osteocytes, and increase strength and muscle. This ultimately leads to increased physical activity and this in turn to activation of bone formation by stimulation of the osteocytes (Notelovitz, 2002).

3.1.3 Progestins and bone

The role of progestins in preventing bone loss is less studied. However, there is general consensus that 19-norderived progestins with androgenic properties, such as norethindrone and norethindrone acetate, at higher doses than necessary for hormone replacement therapy, have beneficial effects on bone density.

Thus, for example, progestins can increase bone density in women with postmenopausal osteoporosis and alleviate the effects of estrogen deficiency in young women treated with GnRH agonists. However, data on the effects of progestins C21 derivatives such as medroxyprogesterone acetate are mixed. It has been reported that, in premenopausal women with luteal defects, medroxyprogesterone acetate (10mg/day, 10 days/cycle) can significantly increase vertebral bone density (Prior et al., 1994). In contrast, administration of 20 mg/day of this progestin could not stop the loss of vertebral bone density in postmenopausal women (Gallagher et al., 1991). In addition, premenopausal women using depot medroxyprogesterone acetate as a contraceptive or taking oral doses (50mg/day) of this progestin on gynecologic pathology have varying significantly decreased bone density (Cundy et al., 1996). The different findings in these studies clearly indicate that the effects of medroxyprogesterone acetate on bone may vary according to the dose administered and the estrogen status of the user. When administered in doses sufficient to induce hypogonadism, medroxyprogesterone acetate is associated with a rapid and significant loss of bone mineral density at the lumbar level. This bone loss is the result of estrogen deficiency and occurs despite an increase in body weight, although it seems partially reversible.

Clinical trials have shown that postmenopausal women receiving norethindrone acetate associated with estrogen show a significant increase in bone mineral density compared with patients treated only with estrogen (Speroff et al., 1996). In contrast, neither the micronized progesterone nor medroxyprogesterone acetate contributed significantly to the positive effects of estrogen on bone (PEPI Trial, 1996).

Progestins influence the bone formation within the bone remodelling process (Sootweg et al., 1992). Receptors for progesterone have been identified in human osteoblasts and

osteoclasts. However, the effects of progestagens on bone are not clear. A study about the activity of a "pure progestogen" on human osteogenic osteosarcoma cells did not observe any effect on cell proliferation when progestins were added alone to culture, but after the combined administration with 17β-estradiol, a strong action synergistically was confirmed on the proliferation of osteosarcoma cells. Moreover, other studies show that some synthetic progestins produce their effects through the activation of the estrogen receptor (Jordan et al., 1993).

3.2 HRT - Type, dose

Almost all information about the effects of estrogens on the bone come from the use of estradiol and conjugated equine estrogens (CEE). Isolated estrone also has a beneficial effect on bone, and it seems that estriol does not have an obvious role in skeletal production in postmenopausal women.

The route of administration, oral or transdermal, does not imply differences in the beneficial effect on bone (Hillard et al., 1994). The estrogenic pulsotherapy has also shown a normalization of the markers of resorption and formation to premenopausal values after 3 months of treatment at doses of 300 mcg/day. The increase in BMD at this dose is similar to that found with 50 mcg/day of transdermal 17-beta-estradiol, providing significant differences in the measurement of BMD over baseline in spine and hip in the evaluation performed after 56 weeks treatment (Palacios et al., 2002). In women with uterus is necessary to administer a progestin to counteract endometrial proliferation induced by estrogen. The dosing regimen of progestin does not influence the beneficial effect of estrogen on the bone so the choice is given by the characteristics of women.

Significant BMD improvements have also been noted with systemic estrogen doses delivered via a vaginal ring. In an randomized controlled trial of 174 postmenopausal women younger than age 65, daily doses of 0.05 and 0.1 mg of estradiol acetate delivered via the ring significantly increased hip BMD (1.7% and 1.8%, respectively) and lumbar spine BMD (2.7% and 3.3%) compared with baseline (Al-Azzawi et al., 2005).

It has been established that a dose range of estradiol between 40 and 50 pg/ml is enough to increase BMD, although a safe level is 60 pg/ml. A dose-response study indicated that daily doses would have more generalizable effect: 0.625 mg of conjugated equine estrogens (CEE), or their equivalents: 0.05 mg of transdermal 17-beta-estradiol or 15 mcg of oral ethinyl estradiol (EE). The standard dose preserves bone mass in at least 80% of postmenopausal women (Table 1). These doses of estrogen and progestogen can induce side effects in both regimes (continuous and sequential), being the most frequent irregular bleeding and breast tenderness. To minimize these undesirable effects, the use of low doses has shown to be also effective in improving menopausal symptoms and quality of life and prevent or reverse bone loss in postmenopausal women (Delmas et al., 2000).

The loss of bone mass and the incidence of vertebral and hip fractures are inversely related to circulating estrogen levels. It has been confirmed that in elderly women estrogen circulating levels of 10 pg/ml improves both BMD and fracture rate. Any increase in estrogen levels has a beneficial effect especially in older women, even when the ultra-low dose is given (25% of the standard dose) (Simon & Snabe, 2007). Neither age nor initial BMD do seem to affect the effectiveness of patterns of low-dose. The effect of low doses of estrogen in women with low BMD has been analized in a randomized, double-blind, placebo-controlled trial, using CEE 0.3 mg/day and 2.5 mg/day of progesterone in women over 65 years and low BMD, in which after 3.5 years of follow up, an increase in vertebral

BMD of 5% and 1.6% in hip has been confirmed, with significant increase in total skeletal and forearm (Recker et al., 1999).

	Low dose	Standard dose	High dose
Estradiol valerate	1 mg	2 mg	3 mg
Transdermal Estradiol	25-37,5 mcg	50 mcg	75-100 mcg
CEE	0,3 mg	0,625 mg	1,25 mg
Estrogen pulsotherapy		300 mcg	
Tibolone	1,25 mg	2,5 mg	

Table 1. Estrogen dose used in HRT

The HOPE study (Women's Health Osteoporosis Progestin Estrogen) (Lindsay et al., 2005), evaluates the effectiveness of low and moderate doses, 0.45 mg/day and 0.30 mg/day alone or combined CEE on vasomotor symptoms, genital atrophy, metabolism, endometrial response and bone density in 2805 women aged 40-65 years treated for 2 years. The results confirmed an improvement in vasomotor symptoms and genital atrophy with low doses comparable to improvement obtained with standard doses, with less bleeding and the beneficial effect on lipid profile. Bone turnover markers such as osteocalcin and N-telopeptide of type I collagen were significantly reduced compared to baseline in the treatment group while no changes were found in the placebo group. BMD increased in both vertebral and hip evaluation. Another study showed similar results (Gambacciani et al., 2001), suggesting that low doses of HRT were able to reduce climacteric symptoms resulting in a decrease in bone turnover and protect against bone loss. The same study showed significant increases in BMD of 2.72 ± 0.3% in women treated while not receiving estrogen therapy had a loss in BMD of 7.9 ± 0.8%.
In an open trial healthy postmenopausal women received for 2 years a low-dose continuous combined HRT containing 1mg estradiol plus 0.5 mg norethisterone acetate, or 0.5 mg of 17-estradiol and 0.25 mg of norethisterone acetate (ultra low dose) along with 1000 mg of calcium per day. The study confirmed that low-dose-HRT and Ultra-low-dose-HRT can alleviate subjective symptoms providing an effective protection against the postmenopausal decrease of BMD (Gambacciani et al., 2008).
Despite the large amount of literature about the beneficial effect of low doses in the bone, there are no studies linking low doses of HRT to the prevention of fractures.

3.3 HRT limitations

Women who may benefit from HRT are those showing climacteric symptoms and also osteoporosis risk. There is general consensus with regard to women with premature menopause should be treated until at least the theoretical age of menopause.
At present there are few absolute contraindications to HRT, being as such pregnancy, vaginal bleeding not studied, active hepatitis, active venous thromboembolism and hormone-specific cancer history.
However, there are circumstances that require careful consideration and consensus with the patient and the presence of pathological conditions such as lupus or endometriosis. Women treated with thyroid hormones or coumarin may need a dose adjustment.

3.4 Duration of HRT

The reduction of bone loss will last as long as you keep the estrogen therapy. When you cease treatment, bone loss returns to pretreatment rate, therefore, to obtain maximum benefit, treatment should begin as early after menopause and stay as long as possible. The optimal duration of treatment has not been fully established, but the results from the Women's Health Inititative (WHI) suggest that estrogen therapy should take the lowest dose for the shortest possible time but then we know that short treatment will not positively affect bone mass.

Analyzing the evolution of bone mass in climacteric women, it has been shown that during the 5 years after the time of onset of menopause the bone loss is equivalent to 60% of what is lost along the climacteric stage; it is at this time when occur the disruption of the trabecular architecture. Once the trabeculae are broken, they will not reconnect again, which would lose bone strength despite getting increases in BMD. Thus, estrogen therapy, at least theoretically, should start as early and stay at least that long in women with natural menopause and up to 55 years in women with early menopause. This would ensure that the loss of bone mass will be delayed, and it will have a positive impact on the possibility of developing osteoporosis and hence on the quality of life.

The use of combined HRT has been associated with an increased risk of venous thromboembolism or coronary artery disease (after a year of use), acute stroke (after 3 years of use), breast cancer (after 5 years of use) and gallbladder disease. Long use of estrogen alone was associated with increased stroke and gallbladder disease. According to recent re-analysis, age is a determining factor in establishing the risks, resulting in very young women (50-59 years) a very low absolute risk (Farquhar et al., 2005).

However, even if treatment is started long after menopause, there are substantial gains in bone mass. In the Framingham study, elderly women with a mean age of 76 years and 7 years since menopause had a significant increase in BMD compared to nonusers, although the effect was less marked in women over 75 years (Felson et al., 1993).

3.5 Estrogen and progestogen combined treatment

The association of progestin does not counteract the beneficial effect of estrogen on bone. It has been reported that derivatives of 19-norethisterone are effective in preventing bone loss even when associated with low estrogen dose (Christiansen & Riis, 1990).

Some studies suggest a greater benefit by associating progestin, while others have failed to show this superiority to estrogen alone. It is possible that the effect has to do with the type of progestin used and it seems that this superior effect is limited exclusively to the administration of compounds of the family of 19-norethisterone. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial, multicenter randomized controlled trial of 875 postmenopausal women with an average age of 56 years, which after 3 years of follow-up BMD increases are seen in lumbar spine (3-5%) and hip (1.7%) with no differences between groups treated with estrogen alone or combined with progestin (The Writing Group of the PEPI Trial, 1996).

The BMD status before the start of hormonal treatment will not influence the effect of it. It has been shown that estrogen administered with and without progestin increases bone mass in healthy women as well and even in osteopenic women with osteoporosis and fractures (established osteoporosis) (Adachi et al., 1997).

3.6 Estrogen effect on BMD

Estrogen administration, associated or not with progestin, has been shown highly effective in the prevention of bone loss in both natural and surgical menopause. It has been shown how treatment with estrogen and progestin can not only maintain but also increase BMD in postmenopausal women, compared to the decline experienced by the placebo group (Christiansen et al., 1981). More than 50 randomized, placebo-controlled studies have shown that estrogen alone or combined with progestin increases BMD, with values ranging from 4-6% in spine and 2-3% in hip, justifying the difference by the different rate of remodelling of these places (Wells et al., 2002).

Estrogen therapy also appears to be effective in patients with established osteoporosis. Women with low bone mass generally have higher turnover and this turnover increases with age. So the best response to estrogen observed in older women or in women with low BMD might result from the suppression of increased bone turnover associated with improvement in the bioavailability of calcium due to improved intestinal absorption of vitamin D. Using CEE double dose (1.2 mg/day) for one year, a double-blind, placebo-controlled study in 21 osteoporotic women showed an improvement in BMD, more marked in the lumbar spine than in femoral neck and an increase in intestinal absorption of calcium (Citivelli et al., 1988). The authors suggest that the beneficial effect is due to inhibition of bone resorption associated with an increased secretion of calcitonin.

A trial of 50 women aged over 75 years and "physical frailty", of which 90% were osteopenic or osteoporotic, who received 9 months of CEE 0.625 mg/day, showed an increase in lumbar spine BMD of 4, 3% (Villareal et al., 2001). The increase was 1.7% in total hip and 2.3% in trochanter 2.3%, these results being similar to those observed in previous studies.

4. Fracture prevention and HRT

Randomized, case control and cohort studies indicate a potential effect of HRT in the prevention of vertebral, hip and forearm fractures in osteoporotic populations.

A cohort study (Cauley et al., 1995) conducted in 9706 women aged over 65 years, and whose main objective was the evaluation of appendicular bone fractures in women treated with HRT, showed that current users who started treatment within 5 years after menopause decreased the risk of wrist fractures (RR = 0.39, 95% CI = 0.24 to 0.64), hip fractures (RR = 0.60, 95% CI = 0.36 to 1.02) and other nonvertebral fractures (RR = 0.66, 95% CI = 0.54 to 0.80) when compared with nonusers of estrogen. HRT is more effective in reducing fracture risk if you start the five years following menopause, and if their use continues for more than 10 years.

A randomized controlled trial of 4 years of follow up in 464 recently menopausal women (Komulainen et al., 1998), treated with 2 mg estradiol valerate and 1 mg cyproterone acetate daily, demonstrated a reduction in risk with RR 0.29 (0.10 to 0.90 CI).

A meta-analysis (Grady et al., 1992) concluded that there is a 25% reduction in risk of hip fracture in postmenopausal women who used HRT. Subsequently, other published meta-analysis (Torgerson & Bell-Syer, 2001) finds that the use of estrogen alone or combined with progestin for at least one year reduces the risk of nonvertebral fracture (RR 0.73, 95% CI: 0.56 -0.94, P = 0.02). In women over 60 years, the effect was less marked (RR 0.88, 95% CI 0.71 to 1.08, p = 0.22).

In women with established osteoporosis (one or more prior vertebral fractures) has also shown a positive effect of HRT in preventing fractures. In a randomized study (Gonnelli et al., 1997), double-blind, placebo-controlled trial conducted in 75 women who were given

treatment with 0.1 mg of 17-beta-estradiol transdermal and oral medroxyprogesterone acetate, 11 days per month, analyzing the BMD, markers of bone turnover and histomorphometric study of iliac crest biopsy, demonstrated a positive effect of estrogen on the parameters analyzed resulting in a reduction given the frequency of vertebral fracture in the treated group versus placebo (RR = 0.39, 95% CI = 0.16-0.95). It can be infered that the number needed to treat (NNT) is 7 women/year to reduce vertebral fracture.

However, other studies have shown no differences between the treatment groups and placebo. The Heart and Estrogen/Progestin Replacement Study (HERS) included 584 women with coronary disease, with an age range of 44-79 years who were treated with 0.625 mg/day CEE and 2.5 mg/day medroxyprogesterone acetate were compared with a placebo group in a follow-up period of 45 months. In this study, there was no beneficial effect of treatment compared with placebo in hip fracture rates or overall rates of fracture in such women not selected for risk factors for osteoporosis and had a low risk of osteoporosis. After 3 years, they published another analysis of these data, reaching the same results (Cauley et al., 2001).

A review of 57 prospective cohort and retrospective case-control studies noted the limited evidence to confirm the anti-fracture efficacy in women who are on hormone replacement therapy (Reginster et al., 2000). Another review (Beral et al., 2002) of 4 randomized studies that included 20,000 women followed an average of 4.9 years, estimated a reduction of fractured neck of femur in 17/1000 users aged 50-59 years, this reduction rising to 5.5/1000 in older women, 60-69 years.

The highest level of evidence on the effect of HRT on fracture was obtained from the Women's Health Initiative (WHI) (Rossouw et al., 2002), This study was carried out in order to assess the main risks and benefits of the combined hormone preparation most commonly used in the United States, 0.625 mg CEE plus 2.5 mg of medroxyprogesterone acetate to health of postmenopausal women. The study included 8506 women in the treatment group and 8102 in the placebo group, with a follow-up time of 5.2 years. This study (The WHI Steering Committee, 2004) shows unequivocally that estrogen with or without progesterone reduce the risk of hip fracture, vertebral and other fractures, the only treatment that has demonstrated this effect in osteoporotic woman, regardless personal risk for fracture.

The reduction observed was similar to that reported in previous observational studies and meta-analysis. The results in terms of fractures indicated that estrogen alone or in combination with progestin reduces the rate of hip fractures and clinical vertebral fractures by one third compared with placebo. Reductions in other osteoporotic fractures and total fractures were also statistically significant.

All types of therapy, route of application and guidelines-beneficial as indicated in the results of a prospective cohort study with more than one million women (Million Women Study Collaborators, 2003), HRT users had significantly more low risk of fracture than nonusers (RR = 0.62, 95% CI = 0.58-0.66).

A meta-analysis (Wells et al., 2002) indicates that BMD measurements are similar when comparing studies of prevention or treatment, estrogen alone or estrogen plus progestin, transdermal or oral, and different types of progestins. The duration and doses of treatment affect the dose effect on BMD.

HRT has a tendency to reduce the risk of vertebral (RR = 0.66, 95% CI = 0.411 to 0.7) and non-vertebral fracture (RR = 0.87, 95% CI = 0.711 to 0.8). The protection against fractures require longer use of HRT. Cross-sectional studies indicate that for hip fracture prevention. Treatment duration should be between 5 and 10 years (Kiel et al., 1987). Swedish Hip

Fracture In Study, a case-control study (Michaëlsson, 1998) on 1327 women aged 50-81 years who had suffered a hip fracture and 3262 controls, current users of HRT have a substantial decrease in fracture risk compared to older users. The RR of hip fracture was 0.35 (95% CI = 0.24 to 0.53) versus 0.76 (95% CI = 0.57 to 1.01). These data indicate that HRT is effective after menopause to maintain protection against fracture but only recent use was associated with optimal protection, since after 5 years without the protective effect of HRT use drops significantly. The beneficial effect is displayed even when treatment is started long after menopause. Thus, in current users, the initiation of therapy nine or more years after menopause provides a reduced risk of hip fracture are equivalent to those women who started early after menopause but who discontinued treatment.

5. Tibolone

Tibolone is a synthetic steroid derived from 19-nortestosterone, structurally similar to norestinone and noretinodrel, first generation nor-derived progestagens. It is described as a selective tissue estrogenic activity regulator (STEAR) because it has specific effects in different tissues after conversion to three active metabolites following oral ingestion (Kenemans, 2004). It has estrogenic, progestogenic and androgenic effects. Estrogenic metabolites act centrally, on the vagina and other tissues and, together with androgenic metabolites, relieve hot flushes and improve energy and sexual well-being (Nathorst-Booszz, & Hammar, 1997; Nijland et al., 2008). On bone, tibolone has estrogenic effect acting on the estrogenic receptor (Modelska & Cummings, 2002).

5.1 Effects on bone

Preclinical studies indicate that tibolone prevents bone loss (axial and appendicular), caused by oophorectomy or low calcium intake in both young and mature rats and in rats with established osteopenia (Yoshitake et al, 1999). Experimental data conclude that tibolone is as effective as estrogen to prevent bone loss secondary to the decline of ovarian function, observed even in osteopenic rats an increase in BMD and femoral and vertebral bone strength, similar to estrogen (Berning et al., 2001).
In humans, the action of tibolone on the skeletal system is also largely mediated by estrogen receptor binding and stimulation of it by some of its metabolites.

5.2 Tibolone and bone turnover markers

In general, studies show that tibolone decreases bone turnover (decrease in the formation and resorption) similar to that obtained with both estrogen in postmenopausal women with normal BMD and in the osteoporotic patient and, returning the process of turnover the existing levels in premenopausal women (Moore, 1999).
Analyzing the effect of tibolone on BMD, it is shown to be capable of inhibiting the decrease and increase BMD compared with placebo or untreated control in both spontaneous and surgical menopause. Like estrogen, we analyzed the results of the use of low doses of tibolone. Low doses of 1.25 mg/day have an effect on the spine and hip similar to that found with standard doses of 2.5 mg/day, in elderly women and women younger postmenopausal (Gallagher et al., 2001). It has proven effective in maintaining BMD in menopausal women at standard doses.

5.3 Tibolone and prevention of fractures

Tibolone (1.25 and 2.5 mg, respectively) increased lumbar and hip bone mineral density to a significantly greater extent than placebo in women with and without osteoporosis (Kenemans et al., 2009), as it was shown with a dose of 1.25 mg/day compared with raloxifene in a study of older osteopenic women (mean age 66 years) (Delmas et al., 2008). The lower dose also reduced the risk of vertebral and non-vertebral fractures in older osteoporotic women (mean age 68.3 years) in the LIFT study (Cummings et al., 2008).

The data described about tibolone, in both experimental and clinical studies about the turnover markers and BMD, tibolone is similar to estrogen, and considering its relationship to fractures, it is conceivable that tibolone may have a similar effect on them.

To understand the effects of tibolone on the incidence rate of new vertebral fractures in postmenopausal osteoporotic women began the Long-term Intervention on Fractures with Tibolone (LIFT) (Cummings, 2006), a multinational, double-blind trial, including 4000 women with tibolone versus placebo with calcium and a duration of 3-5 years. This study indicates the beneficial effect of tibolone on vertebral fractures (RR = 0.59) but has been discontinued by the increased risk of ischemic and hemorrhagic stroke (RR = 2.3 after 2.75 years).

In a randomized, double-blind, placebo-controlled clinical trial (Cummings et al., 2008), they examined the effect of 1.25 mg of tibolone daily on the risk of vertebral and clinical fractures after 3 years and planned to assess the risks of breast cancer, cardiovascular disease, and endometrial cancer after 5 years. During a median of 34 months of treatment, the tibolone group, as compared with the placebo group, had a decreased risk of vertebral fracture, with 70 cases versus 126 cases per 1000 person-years (relative hazard, 0.55; 95% CI, 0.41 to 0.74; $p < 0.001$), and a decreased risk of nonvertebral fracture, with 122 cases versus 166 cases per 1000 person-years (relative hazard, 0.74; 95% CI, 0.58 to 0.93; $p = 0.01$).

5.4 Adverse effects of tibolone

The results of the LIFT study (Cummings et al., 2008) showed that the tibolone group also had a decreased risk of invasive breast cancer (relative hazard, 0.32; 95% CI, 0.13 to 0.80; $p = 0.02$) and colon cancer (relative hazard, 0.31; 95% CI, 0.10 to 0.96; $p = 0.04$). However, the tibolone group had an increased risk of stroke (relative hazard, 2.19; 95% CI, 1.14 to 4.23; $p = 0.02$), for which the study was stopped in February 2006 at the recommendation of the data and safety monitoring board. There were no significant differences in the risk of either coronary heart disease or venous thromboembolism between the two groups.

6. Conclusions

HRT produce increases in BMD at all skeletal sites. The reduction in fracture risk has been documented by data from a meta-analysis, cohort studies and the WHI study.

Estrogen is a therapeutic option for the prevention and treatment of osteoporosis especially in women with postmenopausal symptoms, with consideration of their long-term use increases the risk.

Therefore, treatment should be individualized by assessing the potential personal risks associated with therapy against the expected benefits. In this way, the patient will maintain continuity in the treatment, and will get the benefit sought in the bone.

Tibolone is as effective as hormone replacement therapy (HRT) in treating symptoms and preventing bone loss, and it improves sexuality. It reduces bone turnover and improves

BMD, specially in trabecular bone. Published studies include few patients and have a short duration. Several large, randomized trials have since yielded additional data on tibolone's efficacy and safety profile.

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Osteonecrosis of the Jaw Involving Bisphosphonate Treatment for Osteoporosis

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1. Introduction

Bisphosphonates (BPs) play a key role in the treatment of both primary and secondary osteoporosis on account of their effect on the calcium metabolism in the human body. The administering of BPs reduces the frequency of fractures of the spine, the neck of the femur and the wrist. They also promote bone mass growth in the whole skeleton. In addition, they improve the quality of life of treated patients significantly (Almazrooa & Woo, 2009; Watts & Diab, 2010).

However, during the course of treatment with bisphosphonates it is of importance to bear in mind the possible development of a specific complication, namely osteonecrosis of the jaw. It should be noted that no bisphosphonate-related necrosis is observed in other bones.

Since 2003 when the first cases of osteonecrosis of the jaw following BP administration were described (Marx, 2003), more references to BPs Bisphosphonate–Related Osteonecrosis of the Jaw, i.e. BRONJ, have appeared in the literature, encompassing new and more numerous groups of patients (Durie et al., 2005; Kos et al., 2010; Otto et al., 2011; Ruggiero et al., 2004). BRONJ was initially observed in patients receiving BPs for malignant tumours, bone metastases (most frequently from breast, prostate or lung cancer), and in cases of multiple myeloma (Wang et al., 2007). BPs such as pamidronate and zoledronate were applied intravenously and doses of the medication exceeded many times over the dose used in osteoporosis. Recently, BRONJ has likewise been confirmed in patients with osteoporosis who had received oral alendronate, and in earlier years - etidronate (Magremanne, 2008; Palaska et al., 2009; Watts & Diab, 2010).

BRONJ manifests itself as a necrotically changed, exposed bone with a depleted mucous membrane and often accompanying by inflammation (Peters et al., 1993). It frequently follows a tooth extraction. It occurs more commonly in the mandible than in the maxilla (Kos et al., 2010; Ruggiero et al., 2004).

Osteonecrosis of the jaw can also be triggered by other factors than the administering of BPs (Almazrooa & Woo, 2009). It occurs after: radiation therapy of the facial area, trauma (osteotomy of the jaw bone or during intubation), viral infection (Herpes zoster or HIV), fungal infection with Aspergillus, circulatory insufficiency, local application of chemical agents in dental treatment, inhaling cocaine, and osteomyelitis. Also described is the idiopathic exposure of the lingual surface of the mandibular base in its posterior section in the area of the protruding mylohyoid ridge covered with thin mucous membrane and

poorly vascularised as a physiological result of trauma of the mucosa membrane in generally healthy individuals. BRONJ most frequently appears in this region. Osteonecrosis is also encountered in cases of long-term steroid use, but usually it affects the femur (Almazrooa & Woo, 2009).
Diagnosis of BRONJ can be made in case of bone exposure lasting longer than eight weeks, with no previous history of radiation therapy of the facial region (Almazrooa & Woo, 2009; Ruggiero et al., 2006).

2. Clinical and radiologic profile

Necrosis of the jaw bone in patients treated with BPs can remain asymptomatic for many months or even years.

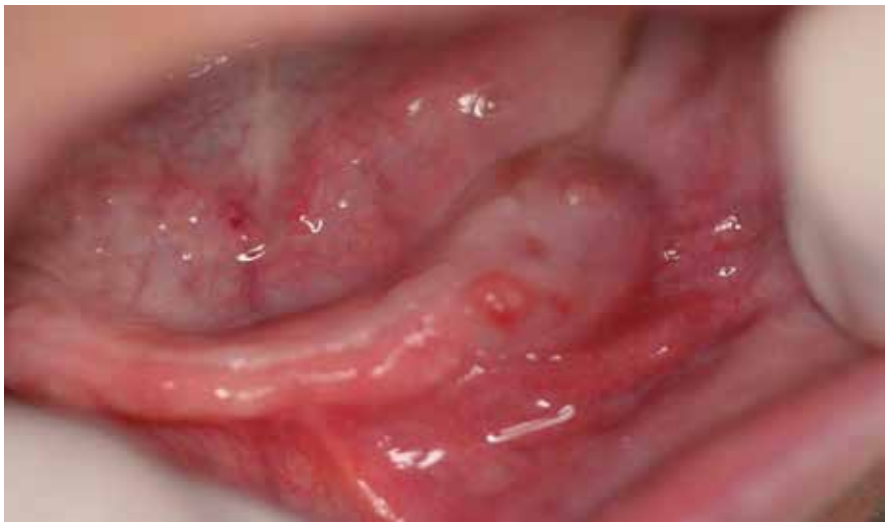

Fig. 1. Redness of the oral mucosa and purulent fistulas of the lower gingiva

The bone becomes exposed, and is sometimes accompanied by pain. The first symptoms before the emergence of a clinically developed image of necrosis include pain, tooth mobility, swelling and redness of the mucosa membrane, and ulceration (Figure 1). These symptoms can appear independently, but much more commonly they do occur after surgery on the alveolar ridge, mostly after tooth extraction. Since the post-extraction socket does not heal, subsequently pain sensation occurs, followed by inflammation of the surrounding tissue and bone necrosis. This leads to pathological fractures of the mandible, the appearance of skin (Figure 2) and gingival (Figure 1) fistulas or secondary inflammation of the maxillary sinus and oro-antral fistulas in the area of necrosis. Numbness of the skin of the lips and face may also be observed. In the initial period the exposed bone is smooth before later becoming rough and coarse. The sharp border of the bone can cause subsequent ulceration of the surrounding tissue exposed to the injury. The most frequent site of traumatic ulceration is the posterior-lateral part of the tongue adjacent to the sequestrum on the lingual surface of the mandibular body (Migliorati et al., 2005; Ruggiero et al., 2006).
Initially radiological images show no significant changes (Figure 3). There may be a widening of the periodontal space around existing teeth, and later rarefaction of the bone as

in the case of bone inflammation, as well as loss of bone structure. Later, bone sequestra may develop, leading to pathological fractures (Figure 4) (Almazrooa & Woo, 2009; Ruggiero et al., 2006).

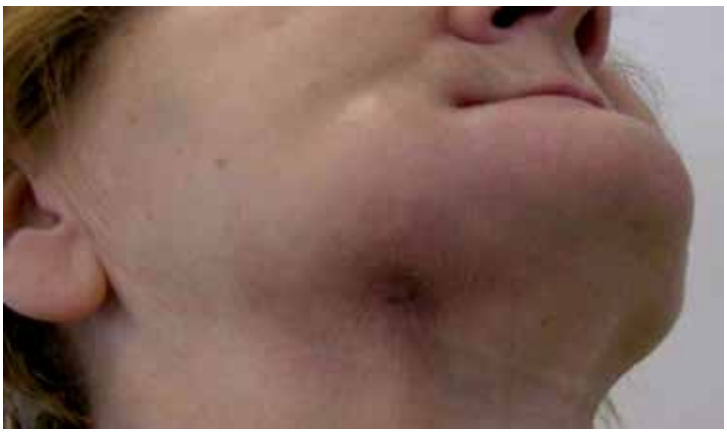

Fig. 2. Skin fistula in course of BRONJ

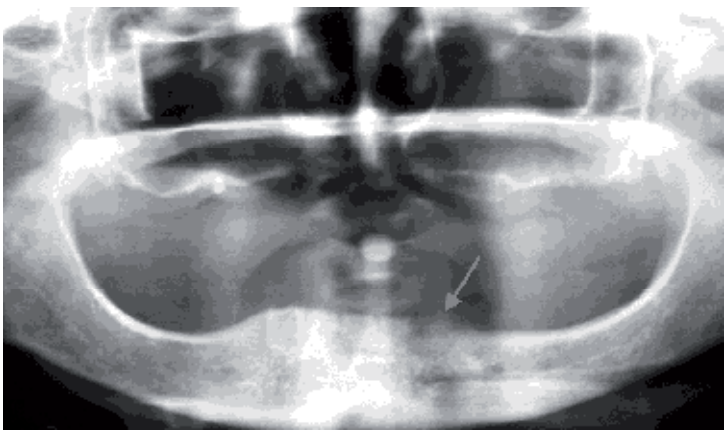

Fig. 3. Unobtrusive marginal osteolysis during the initial phase of BRONJ (arrow)

Histopathological examination demonstrates typical images of chronic inflammation of the bone with fibrous granulation tissue with abundant, chronic partially purulent inflammatory infiltration and necrotically changed osseous trabeculae (Figures 5, 6 & 7) (Migliorati et al., 2005; Ruggiero et al., 2006; Panaś et al., 2010).

Ruggiero et al. (2006) suggested a division of BRONJ into three degrees depending on the progress in the pathology:

Degree 1: Exposure of bone without swelling or redness of the surrounding soft tissue (Figure 1). No change in the radiological image. The exposure of the bone may be preceded by pain.

Degree 2: Exposure of bone with inflammatory swelling of the soft tissue or with a secondary infection, the presence of pain and teeth mobility. Radiological images show necrotic changes in the bone that may resemble rarefaction of bone around the apices of the teeth (Figure 10), widening of the periodontal space.

Degree 3: Exposure of bone with accompanying pain, inflammatory swelling of the surrounding soft tissue or secondary infection that is difficult to control with antibiotic treatments. Appearance of gingival and skin fistulas in the region of bone sequestra or pathological fractures of the mandible, hypoesthesia of the lower lip, as well as secondary inflammation of the maxillary sinus, and oro-nasal fistula in the necrosis of the jaw. Radiograms show bone rarefaction, sequestra, and sometimes pathological fractures (Figure 4).

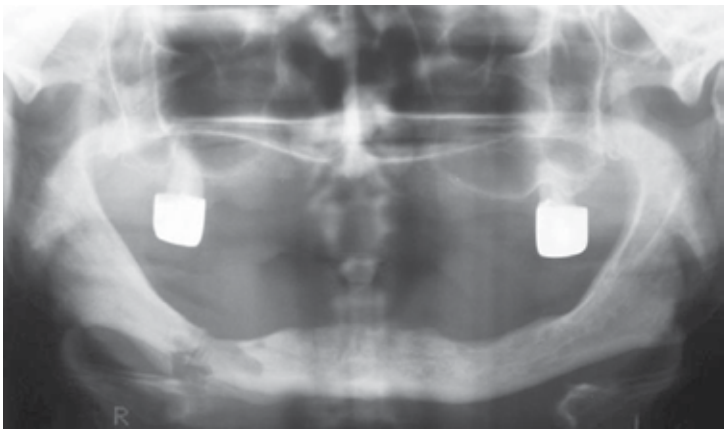

Fig. 4. Pathological fracture in course of BRONJ of the right mandible body

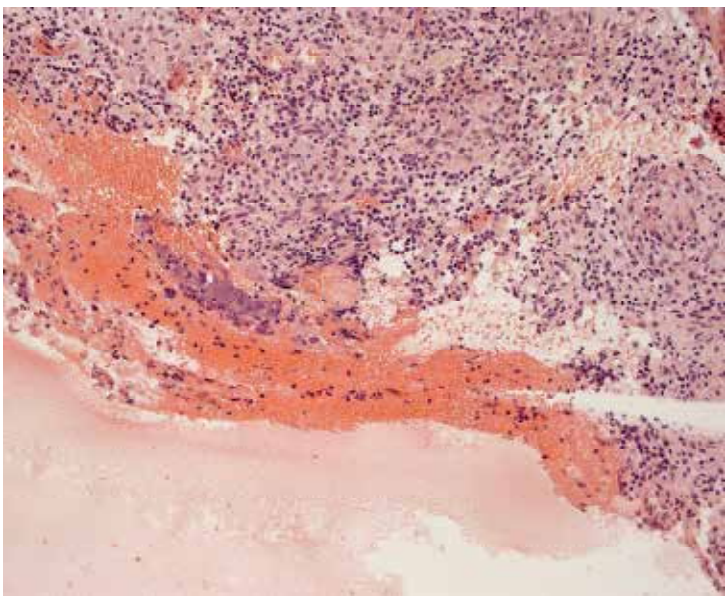

Fig. 5. Histopathological examination of BRONJ (H&E, x60)

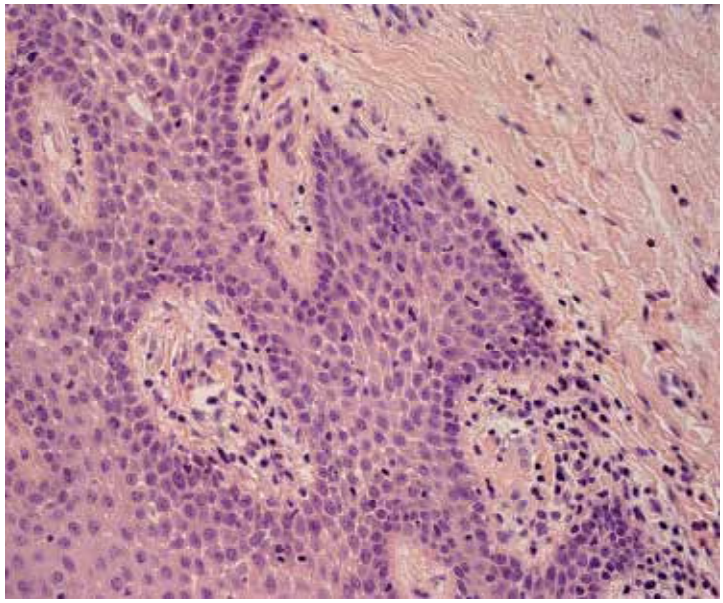

Fig. 6. Histopathological examination of BRONJ (H&E, x120)

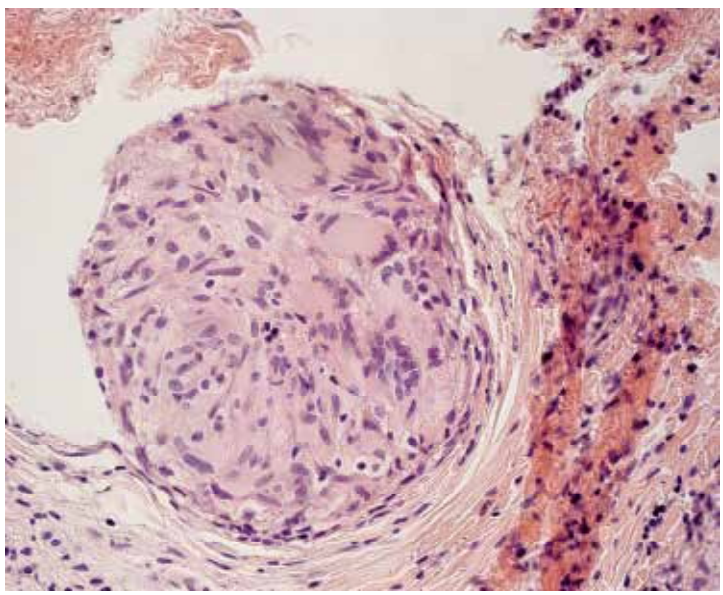

Fig. 7. Histopathological examination of BRONJ (H&E, x140)

3. Pathogenesis

The pathogenesis of BRONJ is connected with the fact that BPs have a significant influence on the physiological process of bone tissue remodelling by hampering the effect of osteoclasts function, leading to their apoptosis and the inhibition of the differentiation of osteoclast precursor cells. BPs also inhibit angiogenesis by reducing the level of VEGF

(vascular endothelial growth factor) in the blood (Almazrooa et al., 2009; Marx, 2003; Ruggiero et al., 2004). Certain bisphosphonates (i.e. pamidronate) significantly reduce blood flow through the diploe, as shown by experimental research, and this may be the reason for the occurrence of ischaemic osteonecrosis (Choi et al., 2007). BPs tend to be deposited in the jaw bones because of their high metabolic rate. The greater metabolic rate is caused by the constant pressure on the bone, especially during chewing. Micro-cracking of the maxillary bone resulting from the physiological force of chewing requires repair, and the need for bone repair and remodelling increases in the case of surgical procedures, i.e. after tooth extraction, which in patients receiving BPs is hampered by the inhibition of key elements in this process (Migliorati et al., 2005). Reduced blood supply in the mandibular body in its posterior section on the lingual side explains the frequent appearance of BRONJ in this area. Risk factors for the development of BRONJ (Table 1) include radiotherapy of the facial region, chemotherapy, the use of corticosteriods, diabetes and coagulopathies (Palaska et al., 2009; Ruggiero et al., 2006). The duration, type and method of BP treatment are also significant factors that determine the appearance of osteonecrosis. The longer the bisphosphonate is administered the greater the risk of BRONJ appearing. When administered intravenously, necrosis occurs 4.4 times more frequently compared to oral route of administration, and among intravenous medications it is most common with zoledronic acid (Almazrooa & Woo, 2009) . The average time for BRONJ to appear is 2 years for BPs administered intravenously among patients with neoplastic diseases, as compared with 4.6 years for oral therapy in cases of osteoporosis, with the minimum time being 3 years (Palaska et al., 2009). Tobacco smoking and excessive alcohol intake also favour the development of BRONJ.

General risk factors	Local risk factors
Concomitant therapies: corticosteroids, other immunosuppressants (eg methotrexate, thalidomide), chemotherapeutic agents (eg hormone antagonists)	Mandibular molar extractions (two thirds of BRONJ cases have been reported in the mandible)
Systemic conditions affecting bone turover: immunocompromised patients, rheumatoid arthritis, poorly controlled diabetes	All dentoalveolar surgery
Smoking	Periodontitis/poor oral hygiene (the bacterial biofil present in periodontal disease is responsible for gingival inlammation and alveolar bone resorption; this pathology, together with the interactions between bacteria themselves and BPs can increase the possibility of BRONJ)
Sociodemographic characteristics: extreme of age (over 6th decade), gender (females)	Trauma related to dentures
	Thin mucosal coverage, lingual to lower molars and bony tori

Table 1. Risk factors for developing BRONJ according to Malden et al. (2009)

A particularly high risk factors for BRONJ development are the extraction of a tooth or any other surgery on the alveolar ridge as well as injury to the mucosa membrane by a denture plate and the occurrence of ulceration (Marx, 2007; Ruggiero et al., 2006). The bacterial environment of the oral cavity favours secondary superinfection with subsequent

inflammation. Aerobic bacteria strains predominate in bacteriological tests, including Streptococci sensitive to penicillin and clindamycin, but Actinomyces species and Eikenella corrodens are also quite often cultured (Block Veras et al., 2008; Panaś et al., 2010). In addition, bacterial products increase bone resorption and decrease rate of bone remodelling, when there is an increased need for remodelling of the bone after surgery performed on the alveolar ridge.

4. Treatment

Treatment of patients with BRONJ is difficult and challenging. The recommended therapy for first degree BRONJ cases is frequent rinsing of the oral cavity with an antiseptic solution, e.g. chlorhexidine, together with regular clinical check-ups of the oral cavity.
In case of a second degree BRONJ one shall undergo antibacterial therapy involving a targeted antibiotic, together with analgesics and rinsing of the oral cavity.
During the third degree BRONJ the surgical removal of necrotic bone is necessary. Targeted antibiotic therapy administered orally or intravenously is also advisable (Rizzoli et al., 2008), as is intensive rinsing of the oral cavity. In addition to the removal of bone sequestra, it is also often necessary to perform a partial resection of the mandible or maxilla (Kunchur et al., 2009; Ruggiero et al., 2006; Williamson, 2009). Moreover, some authors propose the application of hyperbaric oxygen therapy (Migliorati et al., 2005).
Discontinuation of BPs therapy remains an issue of contention on account of their long half-life time, i.e. approximately 10 years, after they become concentrated within the body skeleton (Dello Russo, 2007). Any decision to withdraw BPs due to the development of BRONJ should be made by consensus with the attending physician and dentist.
Due to the long half life times of BPs in the bone, osteonecrosis recurs despite the introduction of the appropriate treatment (Watts & Diab, 2010).

5. Prevention

Because of the difficulties in treating BRONJ and the specificity of this chronic disease, prevention is of vital importance.
Before BPs treatment is implemented, all patients should be referred for dental examination (Shane et al., 2006). It is important to achieve oral cavity assanation, so that no surgical procedures on the alveolar ridge will be necessary during the course of BPs treatment, which significantly increases the risk of the development of BRONJ. It will be necessary to extract those teeth that are not suitable for conservative or endodontic treatment, carry out conservative therapy on other teeth and also perform periodontal treatment. Teeth for which the prognosis for restoration is poor should be extracted. Other essential hygienic procedures and elective dento–alveolar surgery should be performed during this period. The introduction of bisphosphonates should take place 4 - 6 weeks after the dento–alveolar surgery, after suitable healing of the bone wound (Kunchur et al, 2009; Malden et al., 2009; Ruggiero et al., 2006).
Prophylactic procedures in the oral cavity, consisting in the maintenance of good oral hygiene, control of caries, and conservative therapy, must continue for the entire period of BPs treatment. Patients using removable partial or complete dentures must be examined to identify any possible pressure of the denture base on the mucosa membrane of the oral cavity as well as the emergence of decubitus ulcers, especially in the lingual region of the lower prosthesis.
Patients must be taught the necessity of regular dentist check-ups and maintaining perfect oral hygiene as well as the importance of refraining from smoking and alcohol drinking. Patients

should also be made aware of early manifestations of developing osteonecrosis, which should be reported immediately to a dentist; any pain sensations in the oral cavity, oedema or bone exposure, should be reported to the attending physician (Haumschild & Haumschild, 2010).
During the course of BPs treatment surgical procedures involving teeth extractions should be avoided. If a tooth is not suitable for restoration crown of the tooth should be removed, but its roots should be left in place after endodontic treatment. However, significantly mobile teeth with periodontal abscess should be extracted. The timing and conditions of this procedure should be determined by the dental surgeon in consultation with the attending physician. Certain authors suggest that to minimise the risk of BRONJ, BPs treatment should be interrupted ("drug holidays") prior to the planned surgical procedure and, if the need arises, BPs should be replaced with a different medication used for osteoporosis. Recently it has been postulated that the CTX test (the C–terminal Cross–Linking Telopeptide test) should be carried out beforehand. This test can identify a risk group of patients treated with BPs as a measure of the total rate of bone remodelling. A safe CTX value prior to the procedure is 150 pg/ml. The surgery should be carried out with an antibiotic prophylaxis, the most recommended being penicillin derivatives or metronidazole (Bahlous et al., 2009; Kunchur et al., 2009; Malden et al., 2009; Marx et al., 2007).
Placement of dental implants in patients receiving intravenous BPs should be avoided (Ruggiero et al., 2006). However, some authors claim that oral route of BPs administration does not conflict with dental implant placement (Dello Russo et al., 2007). Nevertheless, in these cases, prophylactic antibiotic administration is obligatory and informed consent about an increased risk of implant failure should be provided.
In view of the possible development of BRONJ with prolonged BPs treatment for osteoporosis, the option of BP withdrawal after 5 years should be considered, a fact which shall be decided by the attending physician. Prolonged BPs therapy of more than 5 years should be carefully considered for patients with a high risk of spinal fracture, e.g. those with very low BMD (bone mineral density) (Watts & Diab, 2010).
Some authors claim that bone healing in patients who have been taking oral BPs for less than 3 years is expected to be uncomplicated (Marx et al., 2007). In this period, the accumulation of an oral BP in bone is slowed by its minimal gastrointestinal absorption (Dello Russo et al., 2007). Therefore, a serum CTX is not required prior to oral surgical procedures. However, if the patient relates a history of greater than 3 years of oral BP use or fewer than 3 years but with concomitant corticosteroid or chemotherapy use, a CTX test is highly recommended (Marx et al., 2007).

6. Conclusion

BRONJ complications during BPs treatment appear far more commonly among cancer patients who have received high doses of BPs intravenously. However, until now 200 cases of BRONJ have been observed in patients with osteoporosis (Rondon, 2009). Owing to an ageing population and the growing number of patients with osteoporosis, for whom BPs play a key role in their treatment, more attention should be paid to this problem as well as to learning the risk factors for the development of BRONJ. An important factor affecting the outcome of osteoporosis treatment is co-operation between the attending physician and the dentist.
One example of the development of BRONJ following BP administration is the case of a 70-year old female patient with osteoporosis who was treated with oral bisphosphonate (alendronate group) for 8 years. She reported periodic pain and bleeding in the posterior part of the lower gingiva under the denture base, where a small fistula was identified

together with redness of the mucosa membrane. A pantomographic X-ray revealed rarefaction of the bone of the mandibular body on the left side with a diameter of 3 centimetres (Figure 8). Actinomyces naeslundi were cultured in the bacteriological test. With a targeted antibiotic prophylaxis (clindamycin) curettage was performed on inflamed granulation tissue with minor bone sequestra from the area of the bone rarefaction. Histopathological tests showed fibrous-granulation tissue with extensive partially purulent inflammatory infiltration and necrotically changed osseous trabeculae with adjacent colonies of Actinomyces. Two months before, BP was withdrawn and a preparation of calcium and vitamin D3 was prescribed instead. The pain resumed 8 months after the surgery and an X-ray showed an increase in rarefaction of the bone structure (Figure 9). An antibiotic was used once again and the bone was curetted. Nine months later pain and a purulent fistula appeared in the region of the endodontically treated upper premolar tooth 25, together with a focus of the rarefaction of the bone structure on the X-ray (Figure 10), which was subsequently curetted. Histopathological test: osteomyelitis. The patient is currently in the course of another seven month follow-up without complications. CT does not show any new osteolytic lesions in the jaws (Figure 11).

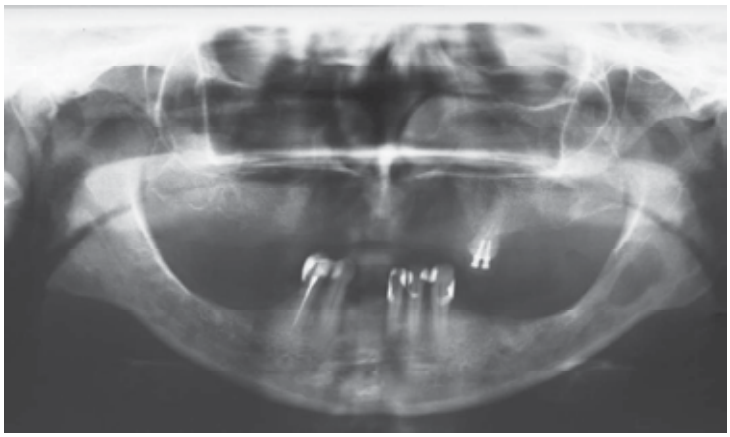

Fig. 8. Focal osteolysis of the left mandible body

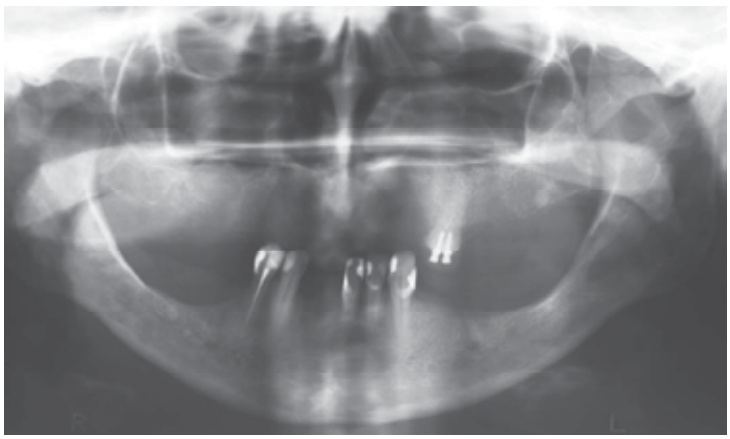

Fig. 9. Follow-up X-ray examination reveals progression of the bone resorption

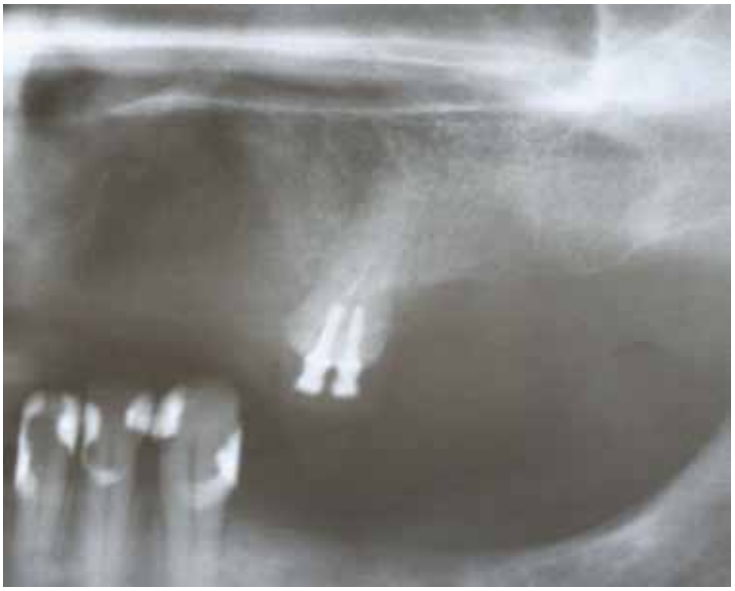

Fig. 10. Osteolytic lesion in the periapical area of the tooth 25

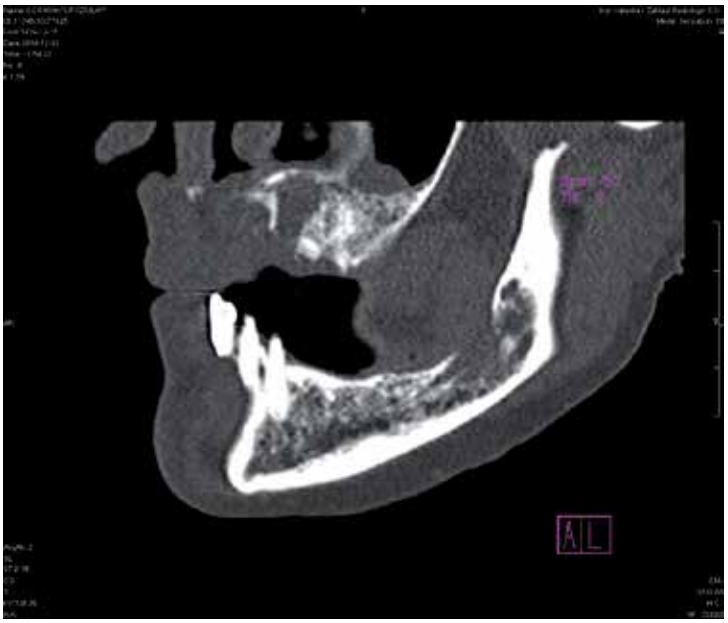

Fig. 11. Follow-up CT-scan confirming the existence of osteolytic lesions in the left mandible body and in the left maxilla. However, it does not reveal any new lesion

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31

Balloon Kyphoplasty for Osteoporosis: Technical Notes

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1. Introduction

Vertebral fractures are the most common fractures in osteoporosis and have significant impact on quality of life and survival rates, as well as carrying increased socioeconomic costs (Becker, S. 2008).
Kyphotic deformity results from those fractures are associated with increased morbidity and mortality (Johnell, O.1996; Edidin AA, et.al. 2011).
Balloon kyphoplasty is a minimally invasive procedure designed to restore vertebral body height, decreasing kyphosis, and also aims to achieve pain relief by stabilization of the fracture (Garfin SR. 2002)
In 1987, Galibert et.al described percutaneous vertebroplasty as an alternative of conventional treatment of vertebral fracture (Galibert P et.al. 1987). The technique and the products used were developed until the description of balloon kyphoplasty in 1998 (Deramond, H . et. Al. 1998).

2. Rational

Balloon Kyphoplasty is performed nowadays by spine surgeons as well as by interventional radiologist. This is certainly an effective treatment of osteoporotic compression fracture, but it's more and more used in traumatic and metastatic fractures (Voggenreiter, G (2005); Wardlaw D. et. al (2009)).
Our experience is based on 176 cases of Balloon Kyphoplasty performed between 2004 and 2010. 136 cases were managed by transpedicular and 40 cases via extrapedicular approach. 82 cases were treated unilaterally and 94 cases were treated bilaterally. Decision for procedure was based on accessibility, extent of collapse and form of the pathological vertebra.

Levels	No. of cases
1	146
2	20
3	6
4	4

Table 1. The number of vertebral levels treated in a single procedure

Most patients were treated for vertebral fracture involving a single level (Table 1).
Fracture types are divided into four groups: Primary and secondary Osteoporosis, trauma and tumor (Table 2).
For secondary osteoporosis, chronic steroid use is the main etiology.

Etiology	No. of cases	% of cases	Cases	Time of the treatment Days
Primary Osteoporosis	156	88.6	10 130 12 4	<10 10-60 60-90 > 90
Trauma	10	5.7	4 2 2 2	2 31 45 50
Tumors	6	3.4	2 2 2	3 50 65
Secondary Osteoporosis	4	2.3	2 2	48 72
Total Number	176	100	176	

Table 2. Etiology and number of patients with chronology of treatment

Vertebral fracture is confirmed through careful correlation of the patient's history, clinical examination, and radiological findings on X-ray's, CT scan and MRI.
The majority of vertebral osteoporotic compression fracture occurs at the thoracolumbar junction: 30 cases at Th12 level and 108 at L1 level (Table 3).
The duration between diagnosis of the fracture and the intervention was between 1 day and 90 days with a mean of 10 to 30 days (Table 4).

Level	Th7	Th8	Th11	Th12	L1	L2	L3	L4
No. of cases	2	4	10	30	108	6	10	6

Table 3. Number of compression fractures cases at each vertebral level

Duration before treatment in Days	1-3	3-10	10-20	20-30	30-40	40-50	50-60	60-90	>90
No. of cases	6	10	52	30	44	10	6	14	4

Table 4. Period of symptoms prior to the treatment of vertebral compression fractures

3. Decision making

3.1 Diagnostic criteria

Patients are eligible for enrolment if they have:

- One to three vertebral fractures from Th5 through L5.

- In case of multiple fractures, at least one fracture needs to have edema assessed by MRI and at least one has to show a 15% loss of height or more in thoracic levels and 10% or more in Lumbar level
- In case of single fracture, it needs to meet both later criteria.
- The fractures treated are not chronic or occurred > 90 days.

3.2 Indication

Indications for balloon kyphoplasty are (Lieberman IH, et. al (2001); Taylor RS, et.al (2007)) :

- Primary or secondary osteoporosis, multiple myeloma, or osteolytic metastatic tumors.
- Painful fractures with a back pain score of 4 points or more on a 0 – 10 scale. And, not responding to conservative treatment for 6 weeks.
- Adjacent vertebra of a fractured and treated one at the level of Th12 or L1 in severely osteoporotic patient, older than 75 years of age with good Karnofsky performance status score (>70). We found that, in 19 cases (7 L1 and 12 Th12 fracture), a fracture of Th12 vertebra occurred in case of previous balloon kyphoplasty on L1 at 18 months and vice versa.

3.3 Contra indication

Patients are excluded if they (Theodorou DJ, et. al (2002); Berlemann, U (2008)):

- Are younger than 21 years of age
- Have chronic fractures (estimated fracture age more than 3 months)
- Have pedicular fracture, previous vertebroplasty of the same vertebra, neurological deficit, radicular pain, spinal cord compression, or canal narrowing
- Are taking uninterruptible anticoagulation therapy
- Have allergies to kyphoplasty materials or contraindications to MRI
- Have dementia
- Are unable to walk before fracture (walking aids were allowed)
- Have vertebral fractures from primary bone tumours, osteoblastic metastases, or high energy trauma.

3.4 Hospitalization and follow up

In our institution, patients are admitted for 24 hours the same day of the procedure. In pre-op, the patient is asked to hold anti-platelets and other anti-coagulation for 5 days. Only LMWH can be tolerated up to 12 hours before the procedure.

Post op, spinal cord X-ray of the operated region is done. The patient is ambulated with abdominal belt for 1 month.

Usually, the patients had regular follow up at 1, 3 and 12 months.

3.5 Biomechanics of cement injection

Injection pressure and cement viscosity are the most important factors for injection. Studies show that the use of more viscous cement in association to lower injection pressure is advantageous for the regular spreading.

For this purpose, a geometrically modified cannulas and high viscosity cement were developed to decrease injection pressure and reduce the risk of insufficient filling of vertebral body (Berlemann,U (2008); Phillips, FM (2003); Vaccaro, A (2003)).

3.6 Surgery
3.6.1 Operative technique

3.6.1.1 Preparation

The procedure is done under local-assisted anesthesia. The patient is in prone position on a radiolucent table in the operating room.
Double C-arm fluoroscopies are positioned. One is for AP view and the other is for lateral view. (Figure 1)

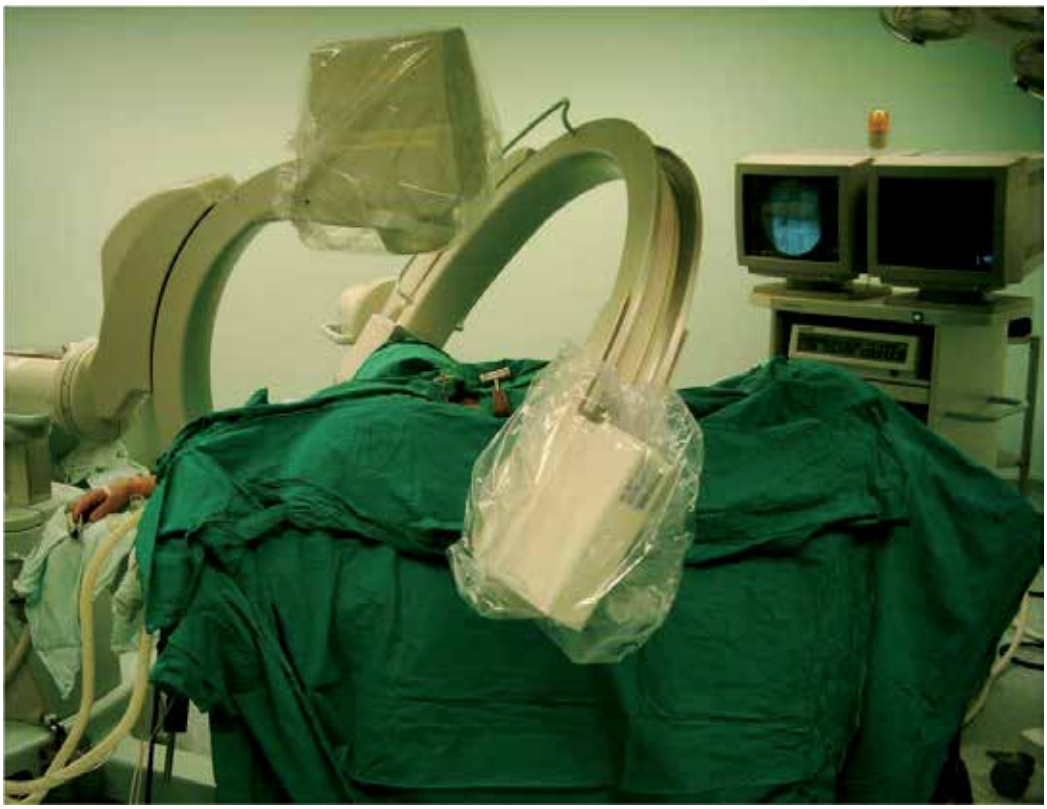


Fig. 1. Position of the patient with Antero-posterior and lateral C-arm.

3.6.1.2 Product used

Polymethylmethacrylate (PMMA) bone cement was the first used in balloon kyphoplasty. It consists of several ingredients that all have their importance. To site, PMMA is composed by methyl methacrylate, PMMA powder ,radio-opacifier, dibenzoyl peroxide, and other additives such as stabilizers, inhibitors, radical catchers, coloring agents and antibiotics (Berlemann,U (2008); Baroud G, Steffen T (2005); Bohner M, et.al (2003)).
PMMA is an easy handled product. It has an adequate balance between high viscosity, which reduces extravasation risks, and low viscosity, which enables low injection forces (Baroud G, et.al (2004); Weißkopf, M, et.al (2008)).
PMMA is allowed to cure for 3 to 5 minutes before injection to achieve a tooth paste viscosity. It needs 8 to 12 minutes in vivo to harden before removing injecting cannulas

3.6.1.3 The instrument used

A basic instrument set with bone access tools is used in the procedure. The set contains the following (Figure 2):

- Scalpel
- Kocher clamp
- Jamshidi needle
- Hammer
- Kirschner wire
- Osteointroducer
- Kyphoplasty balloons with pressure seringue and manometer
- Bone filler
- Cement
- Dermal suture

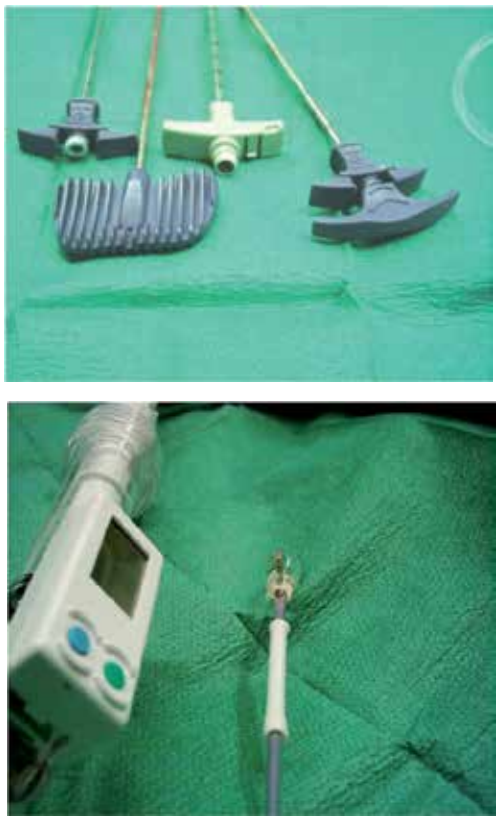


Fig. 2. Above: Jamshidi needle and 2 cement cannulas. Below: Kyphoplasty Balloon with pressure manometer

3.6.2 Operative procedure

The level of the pedicle of the fractured vertebrae is localized under fluoroscopy. A 3 mm incision is made at this level. An 11 gauge biopsy needle is advanced into the fractured vertebral body via Trans or extra-pedicular approach depending on the fracture configuration and patient's anatomy.

A working cannula is inserted over the needle's trajectory. Once it's positioned, the needle is removed. (Figure 3)

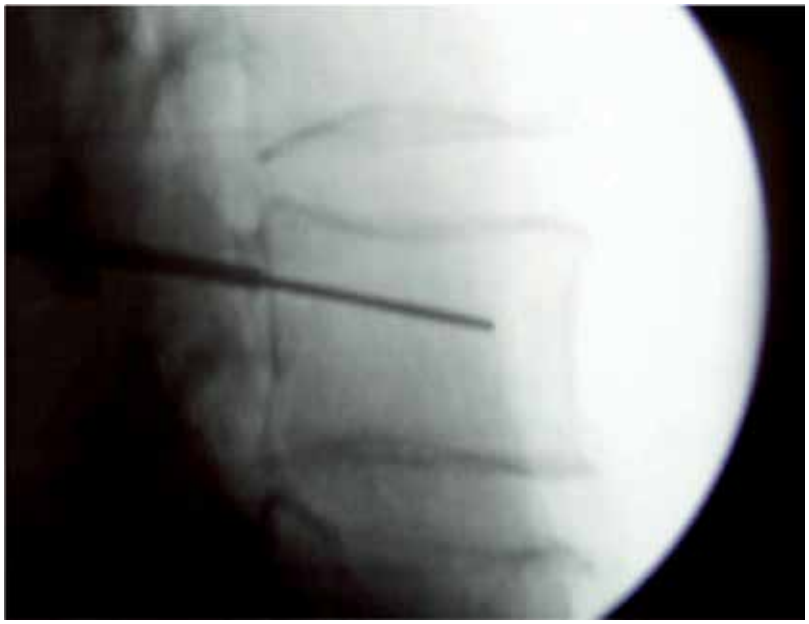


Fig. 3. Insertion of a working cannula.

An inflatable balloon tamp is advanced under the collapsed end plate.
Once inserted through the cannula into the vertebral body, the inflatable balloon tamps are expanded using fluoroscopic control. The volume and pressure are usually managed using the built in digital manometer.(Figure 4)

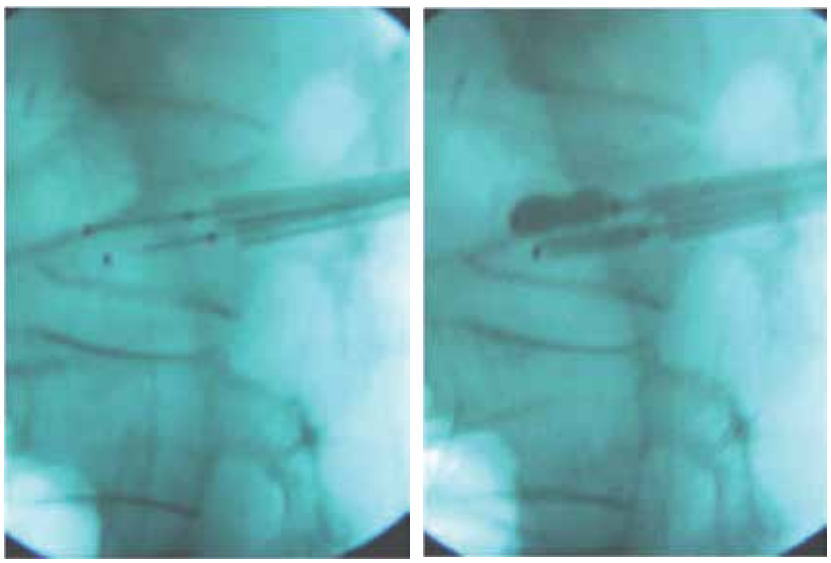


Fig. 4. Right: Insertion of Inflatable Balloon tamps. Left: Inflation of the Balloon tamps.

The balloon is slowly deployed under fluoroscopic guidance until maximum fracture reduction is accomplished. (Figure 5)

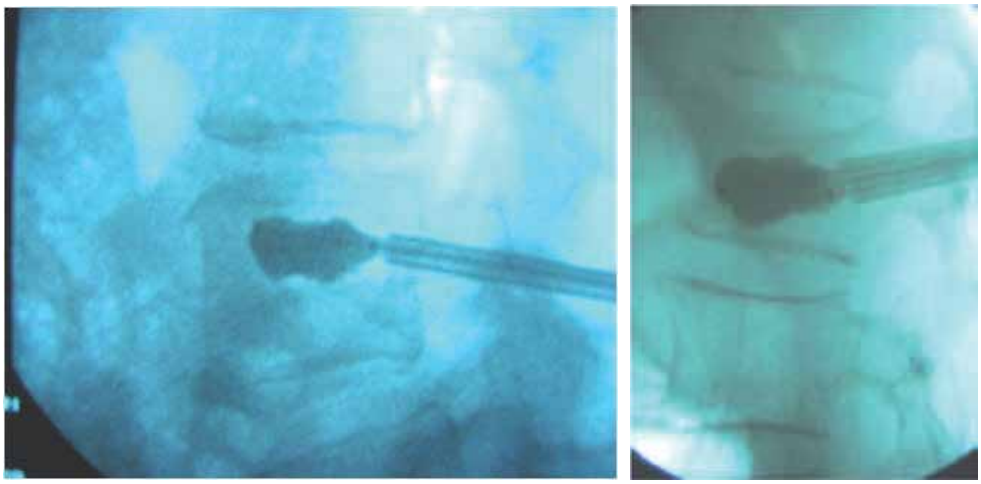


Fig. 5. Maximum fracture reduction under fluoroscopy.

The inflation is stopped when the balloons reaches the cortical wall; Or, when we have "balloon kissing" position in the bi-pedicular approach (Figure 6).
Balloon is deflated and subsequently removed when the cement is ready to be used.

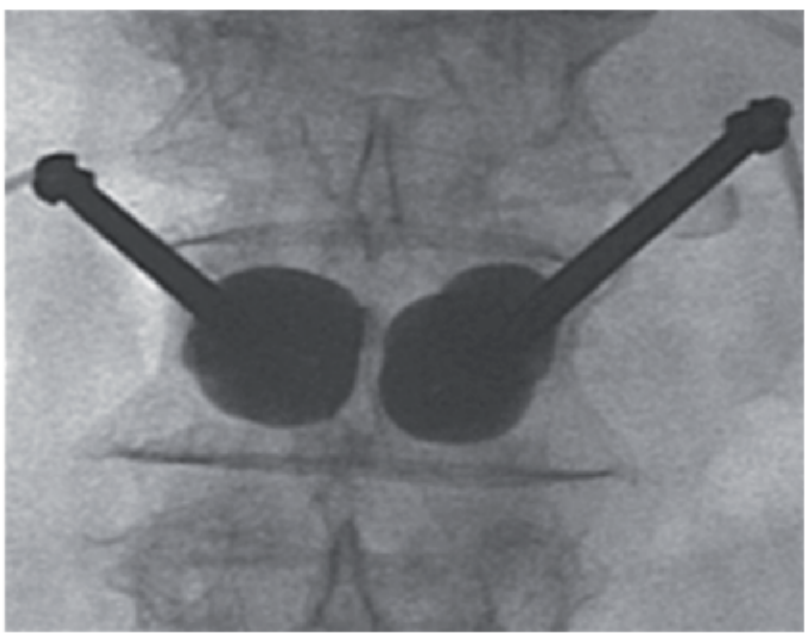


Fig. 6. Bipedicular Balloon tamps inflation, "Kissing Balloons".

During inflation, an assistant prepares the cement to be injected.
The cement is polymethylmetacrelate that needs 5 minutes to reach its semi-solid constitution. It's loaded in 5 injection cannulas

The cement is then injected into the vertebral body's created cavity meticulously under fluoroscopy.
From our observation, the cement fills the entire fracture tract first then it fills the created cavity.
Once packed and hardened, the cannula is removed.
The incision is closed by single cutaneous layer suture.

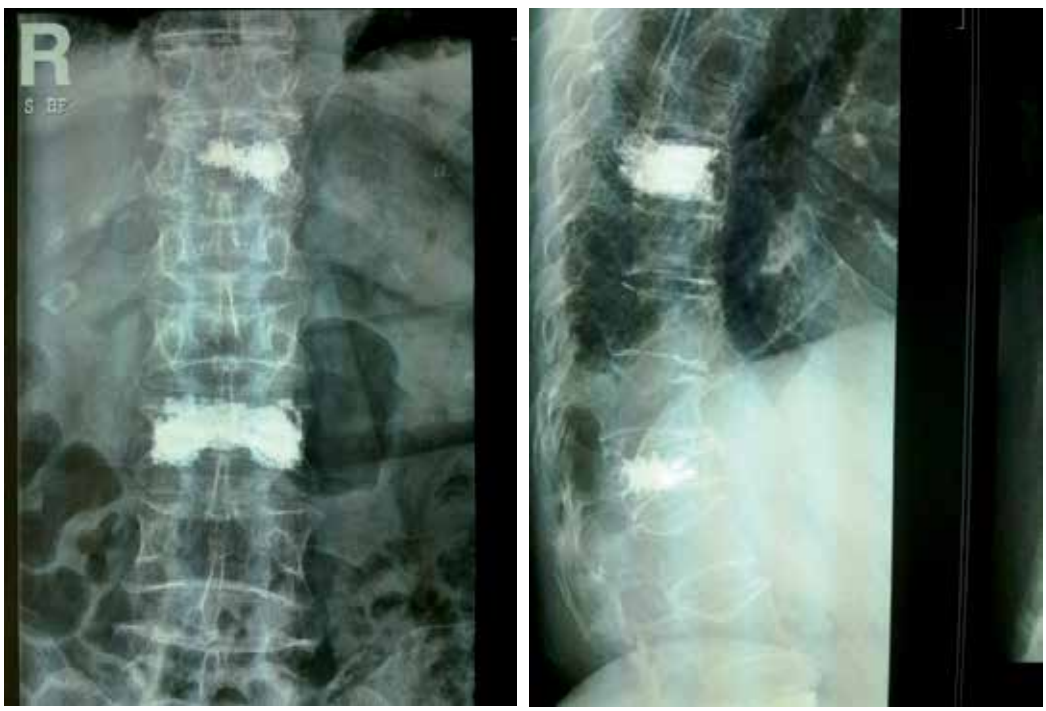
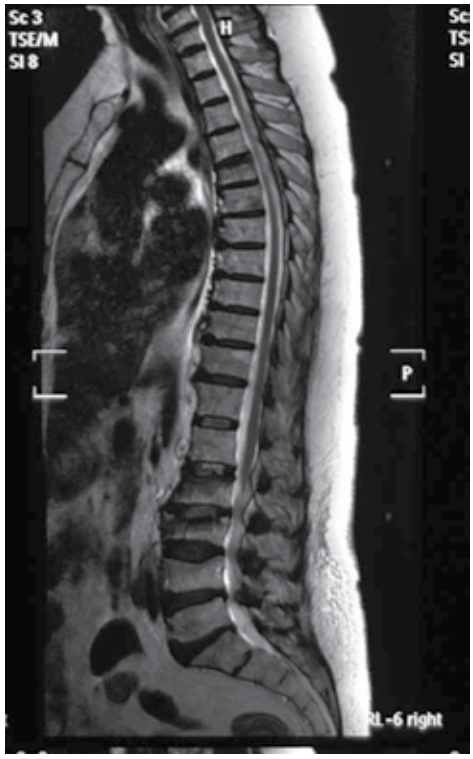


Fig. 7. Antero-posterior and lateral views of a case of 2 levels Kyphoplasty.

3.7 Long term results

As far as the inclusion criteria were respected and for osteoporotic fracture mainly, pain relief was achieved with 24 hours in more than 60% of cases.
The difference of pain relief and kyphotic deformity restoration are highly correlated to the time since the onset of the fracture. And though, patients presenting within 45 days showed optimal results. Whereas, patient presenting after 60 days necessitated moderate potency analgesics to achieve complete pain relief.
Long term follow up, after 3, 6 and 12 months shows an increase in number of cases relieved by the treatment to (70 - 82) % of the population that doesn't necessitate any analgesic use. (6 – 8)%, who already has radicular pain before the procedure, necessitated 3-6 months of Gabapentin treatment.
1% necessitated another surgical procedure and, 9% achieved partial relieve of their pain.



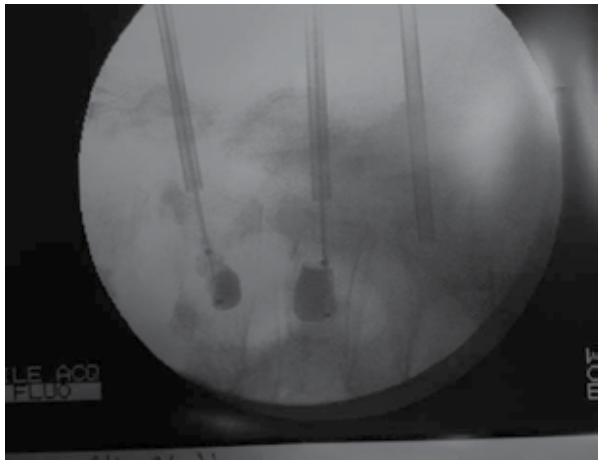


Fig. 8. Above: MRI of the lumbo-sacral spine showing L2-L3-L4 vertebral compression fractures. Below : Per-operative fluoroscopy after balloon inflation and reduction of vertebral height.

3.8 Complications

During balloon kyphoplasty, untoward effect is minimal (3 – 10) %. Minor complications, as cardiopulmonary toxicity defined by transient bradycardia and desaturation, were the most common.

Severe complications as cement pulmonary embolus (Perrin C et.al (1999)), extravasation to the epidural space or to the foraminae are rare (Moreland DB et.al (2001)). The risk of pulmonary embolism is 0.01 – 1%. The rate of extrusions is 8.5%. Nevertheless, most of these extrusions are clinically asymptomatic and the rate of serious problems remains low.
In the literature, the overall clinically significant rate of complication is described as 1%.

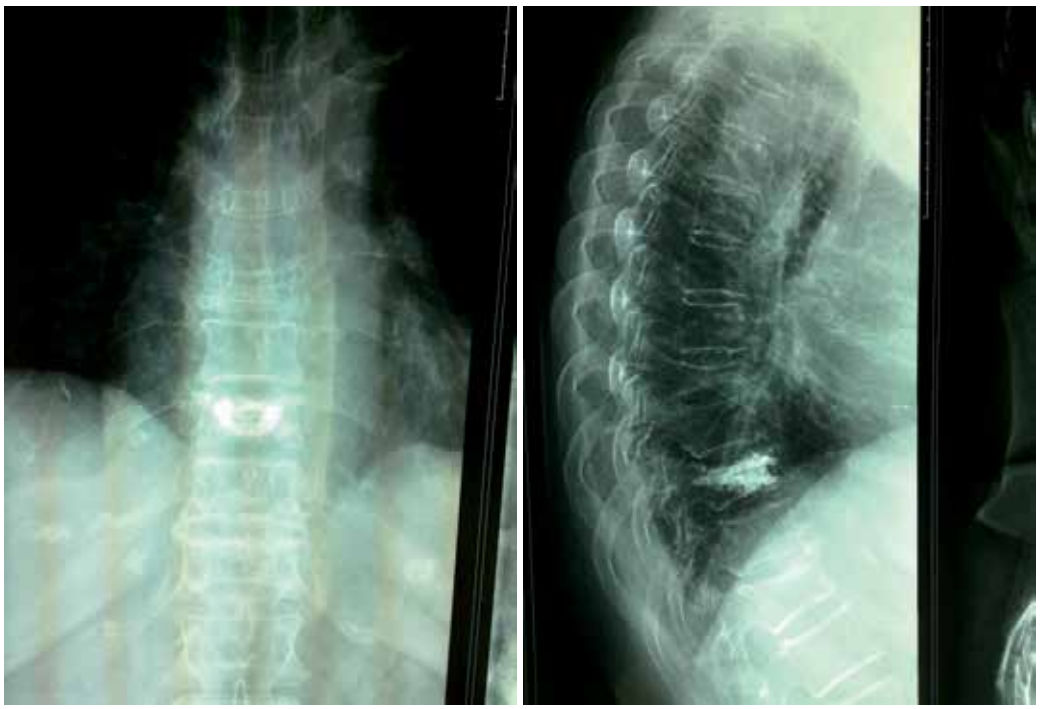


Fig. 9. A case of single level Kyphoplasty.

3.8.1 How to avoid complications

3.8.1.1 Cement embolus

Cement pulmonary embolus are due to hazardous extravasation of the cement to the venous system. The latter is avoided by ascertaining the position of the injector in the middle of the cavity created by the inflated balloon. Clinically, cement pulmonary embolism presents by desaturation with heart rate changes. Thus monitoring is mandatory during the procedure. The procedure should be stopped and management of expected cement pulmonary embolism should be started.

3.8.1.2 Extravasation to the epidural space or foramina

It's the most common complication (Figure 10). Two third of those patients will necessitate open surgical decompression. This complication can be avoided if cement is left to become thick for 5 minutes after injection and the removal of the cannula should be done slowly in rotating manner.

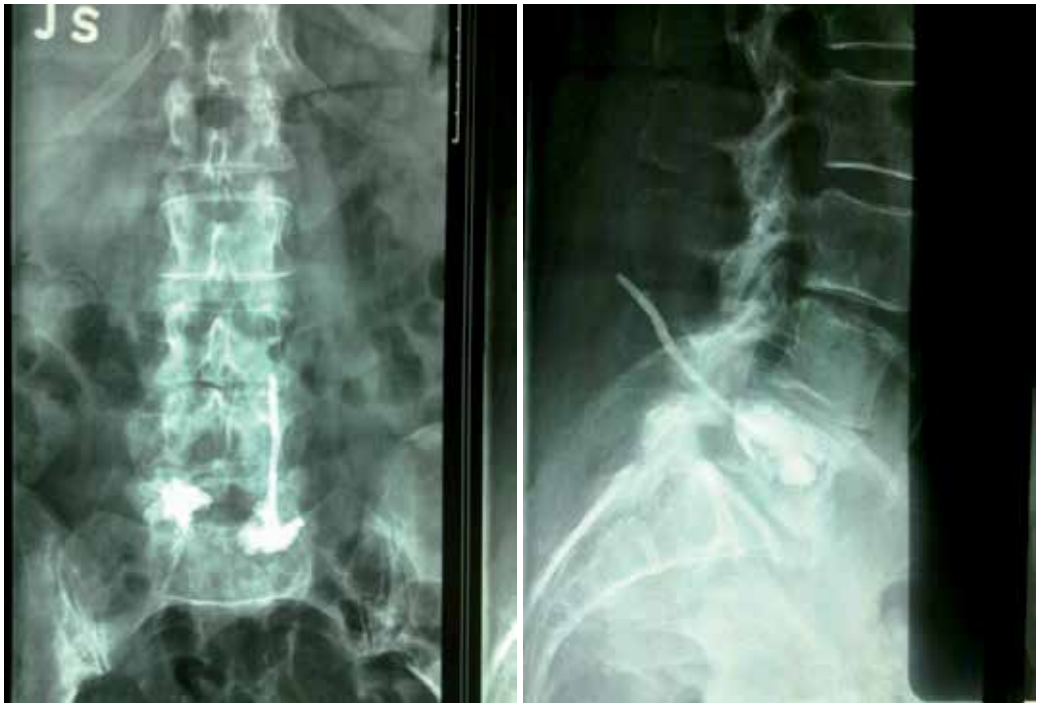


Fig. 10. Extravasation of the cement to the Epidural Space.

4. Conclusion

Despite being relatively a new technique, balloon kyphoplasty becomes popular. It showed, in all studies conducted, that it's an effective method for treating pain and kyphosis induced by osteoporotic vertebral fractures.

With the increase in indications and use of this technique, advancement in injection kits decreased the rate of complications.

In experienced, well trained hands in the field, balloon kyphoplasty is safe and efficient technique for several types of fractures.

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Minimally Invasive Treatment of Vertebral Body Fractures

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1. Introduction

Gestures rather harmless at first sight like weight-lifting , a sudden movement, sometimes only remaking the bed in the morning could cause, in susceptible individuals, the failure and collapse of the vertebral body. Painful fractures, which cause changes in appearance and posture, persistent back pain, limited mobility and a general decay in the affected individuals, most often even unaware of the cause of their evil (Eastell et al., 1991). One of the most frequent causes of fractures of the vertebral body is osteoporosis (Dempster, 2011; Haczynski, 2001), relentless and "silent" disease spread rapidly, due to an aging world population. Vertebral fractures can also be the result of a traumatic event, of hematologic malignancies(multiple myeloma, leukemia), solid tumor metastases to the spine (Bouvard et al., 2011) or long-term steroid therapy(treatment of rheumatoid arthritis, post-transplant patients)(Naganathan et al., 2000). Despite the persistent pain and a more accentuated thoracic and lumbar deformities, those affected often find it hard to realize it , confusing the symptoms with a simple back pain. A compression fracture of the vertebral body not properly treated, increases by 5 times the risk of further fractures, with all that entails in terms of quality of life of the patient and health and social costs (Oleksik et al., 2000). Usually, in case of spinal pain from vertebral fracture, the patient underwent conservative treatment, covering the prescription of a rigid bust, prolonged immobilization and anti-inflammatory medication and painkillers (Prather et al.,2007). A similar solution, however, may not always be sufficient to solve the problem, because the pain can persist for several months and, above all, the patient does not recover the correct posture with increased comorbidity (Cauley et al., 2000) Currently there are minimally invasive methods, such as balloon kyphoplasty, vertebroplasty, and other percutaneous techniques for stabilization, inherently safe for characteristics and dynamics of action, allowing an immediate relief of the pain, and ensuring a good recovery of the statics of the spine (Frank, 2003).

2. Spine anatomy

The spine consists of 33 vertebrae, including 7 cervical, 12 thoracic, 5 lumbar, 5 sacral segments often fused together, and finally 4 coccygeal segments (Gray, 1973). These segments are spaced by the intervertebral discs and structurally connected by ligaments and muscles. Observed in the lateral projection, a normal spine shows lordosis at the cervical

and lumbar regions and a mild kyphosis at the thoracic and sacral regions. These variations in the curvature are important because they are responsible for the orientation of the single vertebra and important components such as the vertebral pedicles, which are the main access route of percutaneous stabilization techniques which will be discussed later(Ortiz & Deramond, 2001). The size of the vertebrae gradually increase from cervical to lumbar tract with variability dependent on the size of the individual. Theoretically there is an increase in volume ranging from 7.2 ml of the cervical area to 22.4 ml of the lumbar spine. In the thoracic area vertebrae are connected bilaterally with the ribs by ligaments that go from the head of the rib to the vertebral body and then from the rib to the vertebral transverse process. The pedicles of the lower thoracic are relatively large and oriented in an anterior-posterior direction. Heading toward the upper tract, we observe a progressive reduction in the size of the pedicles whose orientation becomes more oblique. In the lumbar tract, we observe larger vertebrae and the orientation of the pedicles is different as we go from L1 to L5. The pedicles of the lumbar spine than have a straight anterior-posterior direction similar to that of the lower thoracic. The pedicles tend therefore to be more oblique in the lower lumbar vertebrae reaching their maximum inclination at L5 (fig. n. 1).

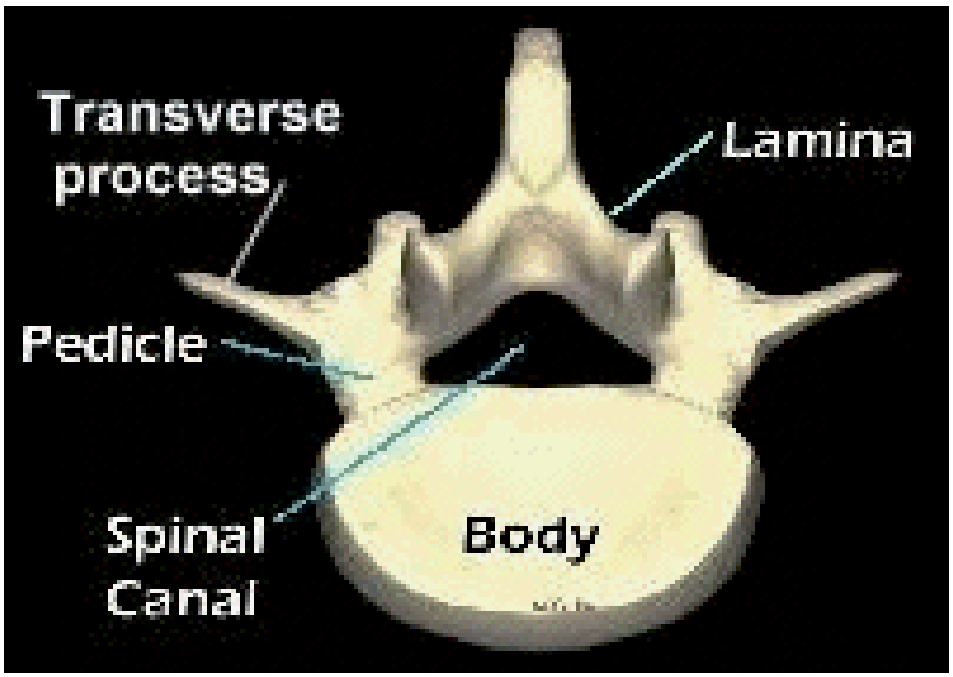

Fig. 1. Vertebral anatomy

The sacrum is then connected to the pelvis through the sacroiliac joints. The sacrum forms the keystone of the pelvis thus counteracting slipping down determined by the higher load . This situation is then responsible for sacral insufficiency fractures secondary to osteo porosis and trauma (De Smet et al., 1985). The blood supply for vertebral bodies derives from arterial branches leaving the aorta , running along the lateral margins of the vertebrae and then sending collaterals to the vertebral bodies, the epidural space and nerve roots. These branches are connected above and below the vertebrae in the paraspinous regions . The

vertebral venous system, instead, consists of three interconnected systems venous valve diameter (interosseous, epidural and paravertebral). These systems are in close communication with the intertrabecular and intraosseous space. The most important venous system, responsible for the drainage of blood from the vertebral venous system, is the basivertebral that connects with the epidural venous system which surrounds the nerve roots and dural sac. The lateral drainage of the vertebral veins communicate with the paravertebral veins forming a system that runs on both sides of the vertebrae vertically and horizontally and interconnects with epidural anterior and posterior venous system . The central veins are major tributaries of the vena cava and azygos carrying the effluent venous blood to the lungs (Groen et al., 2004).

3. Features of vertebral fractures

The vertebral body is made by an extremely thin cortical shell filled with a porous cancellous centrum, the latter carrying about 90% of the load (Duan et al., 2001). During a vertebral compression fracture, the cortex buckles and cracks while the cancellous part collapses and become compacted, reducing the height and volume of vertebra. A vertebral compression fracture (VCF) is defined as a fracture (fig. n. 2) in which there is a partial collapse of the vertebral body with a reduction of at least 20% of the height of the vertebra (Eastell et al., 1991). Vertebral compression fractures may be related to primary osteoporosis, to drugs (prolonged use of steroids as observed in patients with COPD, rheumatoid arthritis, patients with lymphoma or myeloma, transplant patients, androgen deprivation therapy in patients with prostate cancer) or to primary or secondary neoplastic disease. It has been found an incidence of approximately 700,000 spinal fractures annually in the U.S., which represents a large problem for the health care system (US Department of Health and Human Services, 2004), while in Europe every 30 seconds a patient reports a fracture as a result of osteoporosis (EPOS Group, 2002; O'Neill et al., 2009). In Italy each year we observe at least 30 - 40.000 vertebral fractures osteoporosis-related (Johnell et al., 2006) . The disability has been associated with an increase in fee costs for home care and treatment of concurrent medical problems; pain treatment can be difficult: often the pain is not adequately controlled with oral medication alone. It has been calculated that 40% of women of average age (8 out 20) and 15% of middle-aged men (3 out 20) will present one or more osteoporotic fractures during their lifetime (Silverman, 1992). Vertebral compression fractures and the fractures of sacrum have inherent characteristics that are influenced by the biomechanics of each spinal element. VCFs are clearly caused by a number of different force vectors. The intrinsic alignment of the column, the presence or absence of kyphosis or lordosis, has a direct influence on the type of fracture. VCFs in the lumbar and cervical areas are typically determined by a bending mechanism. Since the 3/4 of body weight are distributed in 2/3 of the anterior part of spine, it is common to observe the compression of the anterior part of the vertebral body without involvement of the posterior vertebral wall and the connected elements (Denis, 1983). Vertebral fractures can occur with many simple variants: there may be compression of the posterior wall with or without protrusion of fragments into the spinal canal, whose presence always results in compressive spinal cord or nerve disease; some fractures may lead to the creation of air-filled cavities or liquid in the vertebral body; vertebral compression can be extreme with a loss of vertebral body height more than 70%, ("vertebra plana"). Less frequently, VCFs are caused by trauma, the so-

called burst fractures, characterized by multiple interruptions along the perimeter of the body; less common are those in which there is a separation of the front and the back of the vertebral body (Magerl et al., 1994). It is important to note that the percentage loss of vertebral body height is not related to the amount of pain experienced by the patient nor the duration of pain. The upper endplate is most frequently affected by fractures than the lower one. Most fractures related to osteoporosis are located in the midthoracic (T7–T8), thoracolumbar (T11–T12), and lumbar regions. At the sacrum, rather than vertebral compression fractures, we observe fracture lines that give rise to the so-called sacral insufficiency (Schindler et al., 2007). Fractures can affect one or both wings of the sacrum with or without involvement of the central part. VCFs are associated with significant morbidity with difficulty to perform common activities of daily living and increased mortality directly related to the number of fractures and deformities secondary to changes in the kyphotic spine . These deformities cause respiratory and gastrointestinal disorders. VCFs reduce lung function: a thoracic VCF causes a loss of 9% of forced vital capacity, and lung function (FVC, FEV 1) decreased significantly in patients with thoracic and lumbar fractures (Sclaich et al., 1998). In addition, once an osteoporotic vertebral fracture occurred, the risk of subsequent fracture is increased by 5 to 10 times. In patients with VCF the risk of mortality increases of 23-34%; it has been also observed that in case of hip or vertebral fracture, there is an increased risk of mortality, respectively, from 7 to 9 times (Lindsay et al., 2001).

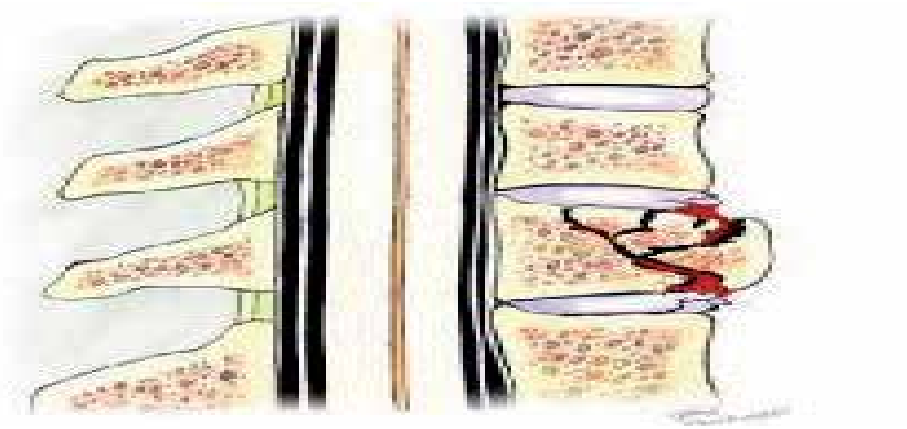

Fig. 2. Vertebral body fracture

4. Evaluation and selection of the patient

Due to the complex etiology of back pain, sometimes the diagnosis of vertebral fracture is delayed despite the persistence of severe back pain and the initial defects of posture not attributable to other causes (Nolla et al., 2001). It has been observed that in most cases the fracture has been recognized during a routine examination. The pain ranges from mild to intense, it can become chronic, but it can also disappear after a few weeks, that is, once the fracture has consolidated. The persistence of pain is higher in people in whom the bone repair is slower. In the acute phase there may be a sudden back pain after a slight injury or no history of trauma; painful is elicitated with local palpation over the posterior elements of

the involved vertebral body, with no radicular pain. In the chronic phase we observe a deformity of the spine, due to loss of height of the vertebral body and the gradual emergence of a protuberant abdomen. The residual back pain in patients with healed vertebral fracture is typically of muscular origin and derives from the now permanent spinal deformity caused by the fracture. In patients with vertebral fracture, in fact, the center of gravity moves forward, creating a wide anterior flexor movement, while muscles of the back and ligaments must offset the increase in flexion. In the presence of contributory causes of back pain (i.e. arthritis and stenosis), treatment of vertebral fracture is not able to offer complete relief from pain.

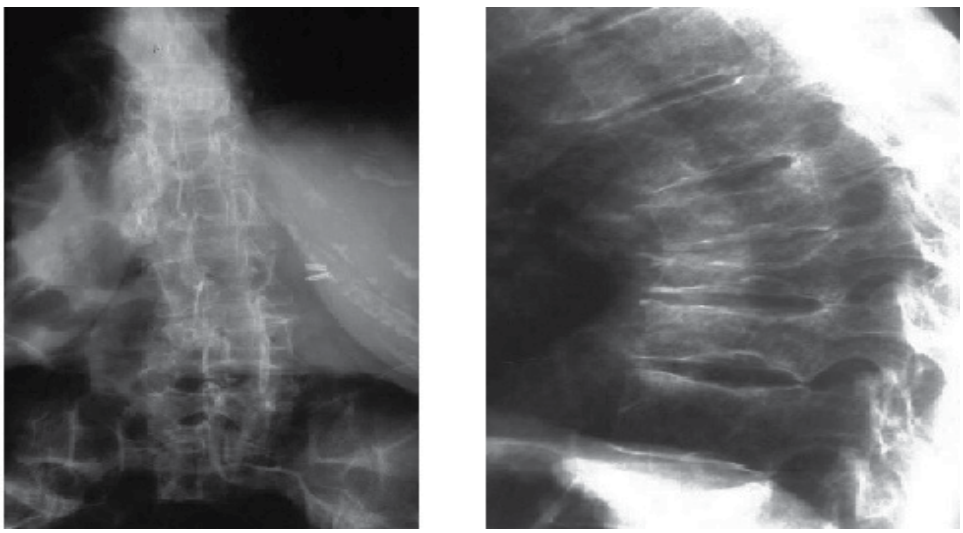

Fig. 3. AP and LL images of dorsal column with a VCF

In order to correctly diagnose a vertebral fracture, we need a thorough neurological examination to rule out concomitant causes and an accurate X-ray imaging (fig. n. 3) . The X-ray imaging includes: preferably a plain radiograph in lateral position as very often the vertebral fracture can be difficult to diagnose if the examination is performed in the anteroposterior position as the direction of the beams is not parallel to the endplates; MRI sequences with T1, T2 and STIR weighted sequences; a CT scan of the affected vertebra; in alternative a bone scan. When the patient with suspected vertebral fracture undergoes an MRI examination, the aim is to look for the edematous reactive component. The finding of bone marrow edema during an MRI is very useful in predicting which patients will benefit most from treatment. Fractures of recent onset, thus with the presence of edema, are those that best respond to the treatment . On sagittal T1-weighted sequences, edema associated with compression appears dark, compared with the high (bright) signal normally seen in the marrow fat. Heavily T2-weighted sequences are the most sensitive, with fluid representing marrow edema; standard T2-weighted fast spin-echo sequences without fat saturation pulse are often insensitive to marrow edema because of the relatively high signal intensity from fatty marrow.

Finally MRI with short tau inversion recovery (STIR) sequences can eliminate all the fatty component to show only the reactive fluid component. In the event that it is impossible to perform an MRI, the patient may undergo a bone scan that identifies osteoblast activity;

unfortunately osteoblast activity has been active for about two years after the fracture and the level of the vertebra with fracture is difficult to identify. CT scan however is mandatory to assess the integrity of the posterior wall of the vertebral body and to assess eventual posterior displacement of bone fragments and eventually to assess the adjacent vertebrae (Wehrli et al., 1995).

5. Techniques of pain relief in vertebral compression fractures

Purpose of the augmentation / stabilization techniques, in case of vertebral fracture, is to obtain adequate pain relief, to ensure the healing of vertebral body so to allow the rapid resumption of activities related to daily life, possibly to restore the height of the vertebral body and thus to counteract spinal kyphosis and the consequences related to it. The spinal augmentation / stabilization techniques are indicated in patients in whom conservative treatment represents another cause of morbidity due to bed rest, immobility and untolerable side effects related to analgesics prescribed, or when an "open" surgical procedure is not advisable on the basis of the patient's clinical condition. The techniques that we will describe are: vertebroplasty(Mathis et al., 2001), balloon kyphoplasty (Taylor et al., 2007) and vertebral stabilization by percutaneous pedicular screws (Foley et al., 2001) . Biplane fluoroscopy allows the procedures to be performed more rapidly, but they can also be accomplished safely with a single-plane C-arm; CT has been described as an aid to fluoroscopy, but it adds considerable complexity and cost to the procedure without corresponding benefit to the routine treatment of a VCF (Gangi et al., 1994).

5.1 Vertebroplasty

Vertebroplasty (VP) was performed as an open procedure to improve the grip of pedicle screws in spinal surgery or during filling of the continuous solutions in the vertebral body after resections for cancer. Percutaneous VP was performed for the first time by Galibert and Deramond (Galibert et al., 1987) for the treatment of severe neck pain secondary to a hemangioma that affected the entire body of C2; after an intervention of laminectomy and resection of the neoplastic component invading the epidural space, they decided to strengthen the structure of the vertebra by the injection of polymethylmethacrylate (PMMA) by anterolateral percutaneous approach. The amount of PMMA injected was 3 ml, with a complete pain relief .The technique was then introduced in the U.S., where it was used primarily to treat pain from osteoporotic vertebral fracture (Deramond et al., 1998). After other experiences, the same authors established key points for the execution of this technique (Mathis et al., 2001). They decided to use large- bore needle (10-13 gauge) for the thoracic and lumbar levels and a smaller needle (13-15 gauge) for the cervical level; the PMMA was made opaque by the addition of contrast to make it visible when injected and to evaluate the distribution during the injection. After a small skin incision, the disposable bone needle is advanced, under fluoroscopic guidance, using an unilateral or bilateral transpedicular/extrapedicular approach (at lumbar and thoracic spine level respectively) through the centre of the pedicle (fig. n. 4) , and then into the vertebral body with the expectation that the central portion of the vertebra can be filled. Fluoroscopy, with frequent switching between the frontal and lateral projections, ensures that the needle is correctly positioned. The tip of the needle should be placed within the anterior one-third of the vertebral body, close to the midline; biopsy, if indicated, can be performed before final

needle placement. Once the needle has been inserted into vertebral body, the cement (polymethylmetacrylate-PMMA) is prepared and mixed until it becomes like toothpaste and then injected trough the needle (between 3 – 6 ml) under continuous lateral fluoroscopic control in order to observe and prevent any cement leakage. The cement diffuses into space and tends to solidify in 1 hour, stabilizing the vertebral body. After procedure, in fact, the patient must remain lying down for several hours, to prevent movement of cement that is not yet consolidated. The approach to the cervical vertebrae is anterior; needle introduction should preferably be done on the right side (opposite the esophagus) and avoiding carotid artery, internal jugular vein, vertebral artery and esophagus.

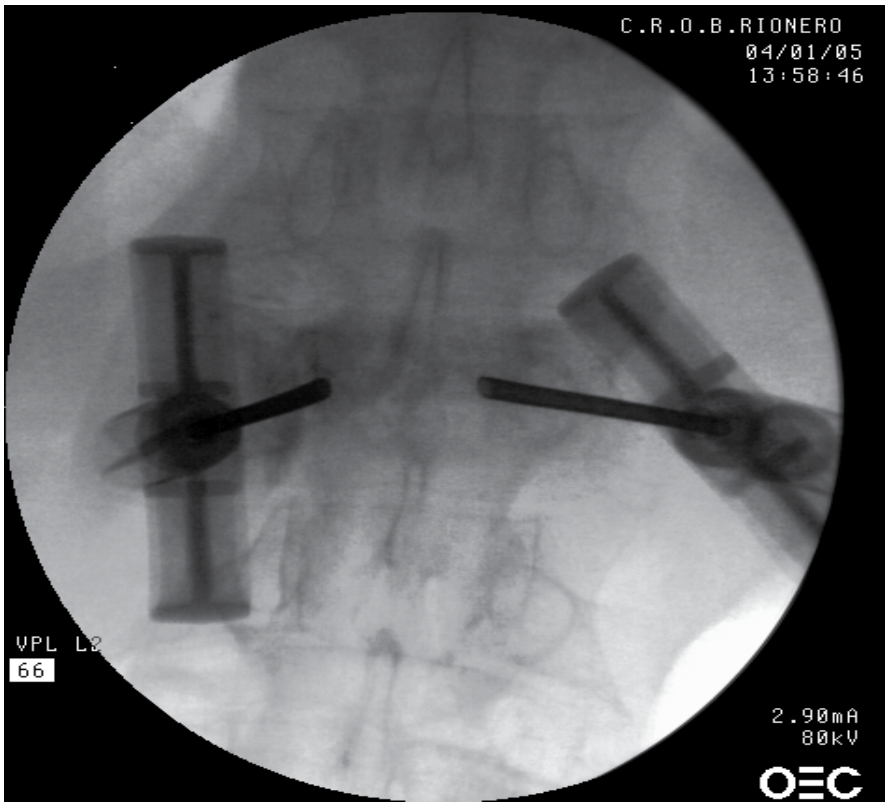

Fig. 4. AP X-ray image of vertebroplasty

Vertebroplasty is a treatment used to get relief from pain, but has little or no effect on the recovery of the height of the vertebral body fractured. The mechanisms, by which we obtain adequate analgesia, are two: the first mechanism is based on the ability of PMMA to combine the individual bone fragments in a single block, avoiding the painful micro shiftings of individual fragments between them. The second mechanism may be related to the exothermic process that accompanies the polymerization of PMMA and that would result in a "thermal neurolysis" of the nerve within the vertebral body. In addition, the PMMA results in a significant strengthening of osteoporotic bone, reducing the risk of subsequent fractures. The incidence of complications ranges from 1 to 3% in osteoporotic vertebrae.

The majority of complications could be divided into:

- minor:
  - bleeding of the site of needle insertion,
  - rib fracture ,
  - transient fever
  - transient worsening of pain symptoms secondary to the heat produced by the polymerization of the cement,
  - cement leaks into the disk or in paravertebral soft tissues
  - new fractures in adjacent vertebrae (Lindsay et al., 2001)
- moderate
  - irritation of the nerve trunks,
  - cement leak in epidural space
  - needle displacement
  - infection
- severe
  - cement leaks into paravertebral veins, leading to pulmonary embolism, cardiac perforation, cerebral embolism and even death. (less than 1% when treating osteoporotic compression fractures, increasing to 2–5% when treating osteolytic metastatic disease) (Scroop et al., 2002).

The possible extrusion of cement in the spinal canal (which occurs with an incidence of 3%) is a feared complication, requiring immediate surgical decompression in an attempt to limit the damage from spinal cord compression (Mathis, 2003). Cement can also leak into the disk space. We do not know actually if a cement leak into the disk may be responsible for fracture of an adjacent vertebra as adjacent-level fractures after VP are known to occur also without leak.

After vertebroplasty, it has been reported a marked improvement in pain symptoms in 90% of cases, but residual pain may persist in the early days, in the area of needle insertion or for muscle distraction. The complete disappearance of pain, accompanied by the discontinuation of analgesic drugs has been observed after 3 to 6 weeks. Despite the disappearance of pain, the patient must pay attention to physical activity as the possibility of subsequent vertebral fracture is always present.

5.2 Kyphoplasty

Kyphoplasty (KP) has been introduced as an alternative approach in US (Garfin et al., 2001). It can be performed in thoracic vertebrae from T5 to T12 and on all lumbar vertebrae. It is similar to vertebroplasty and has been referred to as "balloon-assisted vertebroplasty" (BKP). Kyphoplasty is a technological advancement of vertebroplasty (fig. n. 5); beside the relief of pain secondary to the VCF, it is possible to obtain a partial recovery of the height of the vertebral body (Lieberman et al., 2001). To restore vertebral anatomy after a fracture, the vertebral endplates must be reduced to their correct anatomic position. This action requires the volume of vertebral body to be increased (creation of a void) and requires sufficient separing force to move the endplates (reduction). The reduction of the fractured vertebra reduces the kyphosis of the spine; this effect determines an esthetic improvement (posture) and could reduce the risk of fracture of the adjacent vertebra as a result of abnormal load bearing. Kyphoplasty entails the inflation of a percutaneously delivered balloon in the

vertebral body; the balloon restores the vertebral body height in addition to creating the cavity. Into the cavity created by the balloon, a preparation of PMMA thicker than that used in vertebroplasty is then injected under relatively low pressure; because this PMMA is more viscous that used for vertebroplasty and it is injected under lower pressure that in vertebroplasty, the risk of intravascular extrusion is thought to be lower. The risk of cement extravasation is reduced due to containment produced by the newly created vertebral cavity. The entity of the vertebral body reduction varies from case to case, depending by the maximum volume of the balloon inflated and the pressure required to .Although associated with a finite level of cement leakage, serious adverse events appear to be rare. Osteoporotic vertebral compression fractures appear to be associated with a higher level of cement leakage following BKP than non-osteoporotic vertebral compression fractures (Taylor et al., 2007).

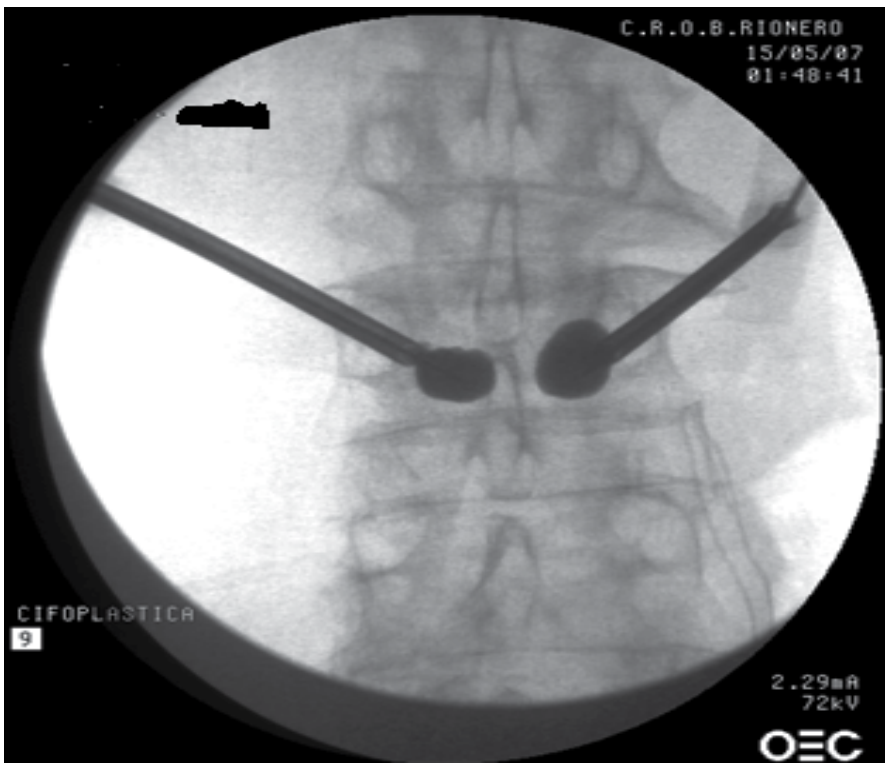

Fig. 5. AP X-ray image of kyphoplasty

In 2009 it has been conducted a study in which 300 patients have been randomly assigned to receive kyphoplasty treatment or non-surgical care. The primary outcome has been the difference in change from baseline to 1 month in the short-form (SF)-36 physical component summary (PCS) score between the kyphoplasty and control groups. Quality of life and other efficacy measurements and safety have been assessed up to 12 months. Serious adverse events (such as myocardial infarction and pulmonary embolism) did not occur perioperatively and were not related to procedure. Authors concluded that balloon kyphoplasty was an effective and safe procedure for patients with acute vertebral fractures and could be used as an early treatment option (Wardlaw et al, 2009).

5.3 Cement selection

The introduction of an external component in the human body brings up the general problem of biocompatibility. Several types of cement are actually available: the recent development of polymethyl metacrylate cement (**PMMAs)** and the market introduction of new cements like **composite cements** and **calcium phosphate cements,** allow physicians to choose the best material for the treatment of different lesions causing vertebral pain.

5.3.1 Polymethyl methacrylate (PMMA)

The most commonly used cement is poly-methyl methacrylate (PMMA) and its function is to immobilize the fracture and increase the strength of the vertebra. PMMA cements fall into two general categories: rapid set or slow set types. Most inexperienced operators initially feel more comfortable by using the slow-set varieties, because these materials allow more working time of the cement at room temperature; however, the rapid-set materials offer definite advantages that quickly surface. A new acrylic osseous cement, with 10% hydroxyapatite well-known for its osteo-conductive properties, possesses better biocompatibility than traditional cements. The hydroxyapatite particles on the surface of the cement improve the response from the osteoblasts, consequently reducing inflammatory reactions . The high viscosity properties of Confidence Spinal Cement System ©(DePuy Spine, Inc 2011) allows for interdigitation, preserving the trabecular structure of bone; this cement shows immediate post-mixing high viscosity, so reducing the potential leakage within vertebral body. *N*-methyl-pyrrolidone (NMP) has been added to a PMMA bone cement (Boger et al., 2009) making the PMMA cement more compliant for the use in cancellous bone augmentation in osteoporotic patients due to modification of its mechanical properties similar to those of cancellous bone, a lower polymerization temperature, and an extended handling time.

5.3.2 Composite cements

They have been used since the late 1970's in orthopedic applications, like pedicle screws augmentation. Those cements offset the disadvantages of PMMA like the exothermic reaction, the release of unreacted monomer in the circulatory system and the modification of the initial composition of the PMMA (changes in the monomer-to-polymer-ratio and addition of contrast materials).Moreover they appear to be more biocompatible, easy-to-handle with sufficient radiopacity and with good biomechanical properties. One of these composite cements is Cortoss® (Sun et al., 2008) developed by Orthovita-Malvern, USA, a glass-ceramic reinforced cement based on the Bowen molecule diluted with triethylene glycol dimethacrylate (TEGDMA) (Smit et al., 2008). The optimal temperature of Cortoss® to be used is as close as possible to 20°C. Higher temperature will reduce the setting time; to obtain a good fluoroscopic visualisation, there is no need to modify Cortoss, as it contains over 65% of radiopaque fillers.

5.3.3 Calcium phosphate cements (CPCs)

Calcium phosphate cements (CPCs) are made of different calcium phosphate (CaP) powders and an aqueous solution belonging to the category of the low-temperature cements. CaPs are very similar to the mineral part of bone. They are less injectable if compared with to other PMMA cements, which are hydrophobic and tend to stay compact within the vertebral bodies. In order to prevent this problem, we could create a cavity in the vertebral body with an expandable balloon and filling the new cavity with CPC or removal of bone

marrow from the vertebrae using a suction device and injection of the CPC. CPCs are justified in the treatment of recent burst fractures of thoracolumbar vertebral bodies in young patients (Bohner et al., 2005).

5.4 Pedicle screw-assisted spinal stabilization

The use of pedicle screw-assisted spinal stabilization(Foley et al., 2001; Fuentes et al., 2010) has become popular worldwide; pedicle screw fixation is a safe and effective treatment for many spinal disorders, including vertebral fractures not suitable to be treated by vertebro /kyphoplasty. Standard "open " techniques for pedicle screw placement have been associated with a wide median incision of the back and the disconnection of large muscle areas, to allow adequate visualization of the spine and bone, for easy access, with extensive blood loss , lengthy period of hospitalization and costs. Recently it has been introduced into the market a minimally invasive posterior fixation of the lumbar spine in which percutaneous screws and rods are used,minimizing paraspinous tissue trauma without sacrificing the quality of spinal fixation (fig. n. 6).

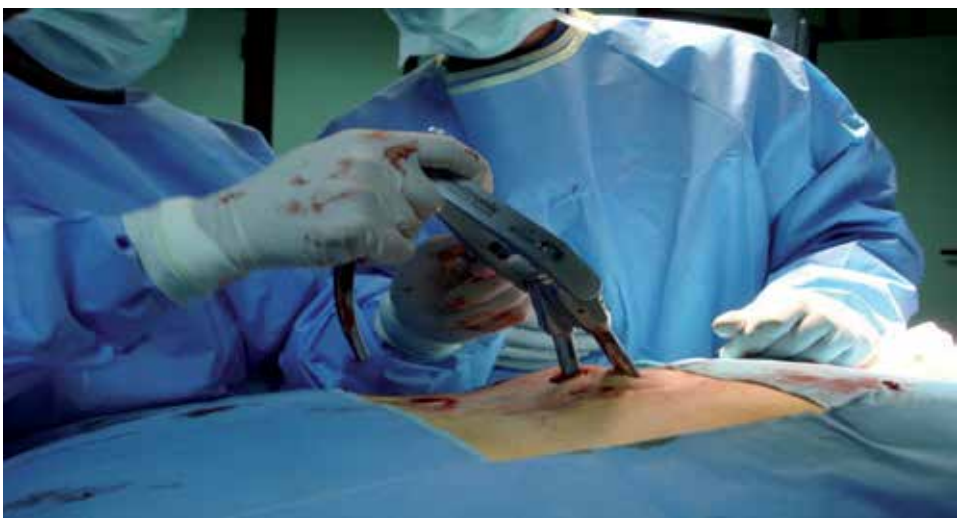

Fig. 6. Live insertion of percutaneous screw assisted spinal stabilization device

In fact the minimally invasive techniques with the aid of new fluoroscopy generation allows to place percutaneous spinal instrumentation accurately , through small skin incisions and with minimal radiation exposure. The vertebral pedicles represent a very strong connecting structure in the spine, so the placement of a screw inside the pedicle allows for a significant strengthening of the vertebra. The length of screws varies according to different dimensions of the pedicles. The most common screws are made of titanium and are equipped with a head (poliassial screws) that can rotate so as to adapt to different conditions and anatomical locations. Once placed, the rods can be percutaneously inserted into the screws, contributing to the stabilization of the spine (fig. n. 7) . The benefit of percutaneous intervention is evident because the surgical incisions are less painful, blunt dissection and the muscle dilation do not alter the normal anatomy, blood loss is minimal, the scars are esthetically irrelevant and hospital stay is significantly reduced. Although there are still not many prospective randomized studies comparing conservative treatment versus mini-invasive methods of

vertebral stabilization (Kallmes et al., 2009; Buchbinder et al., 2006; Clazen et al., 2010; Clark et al., 2011), pain relief is often achieved in 80% of cases with the latter within a few hours , stopping the progression of the deformity of the spine, even in long-term studies.

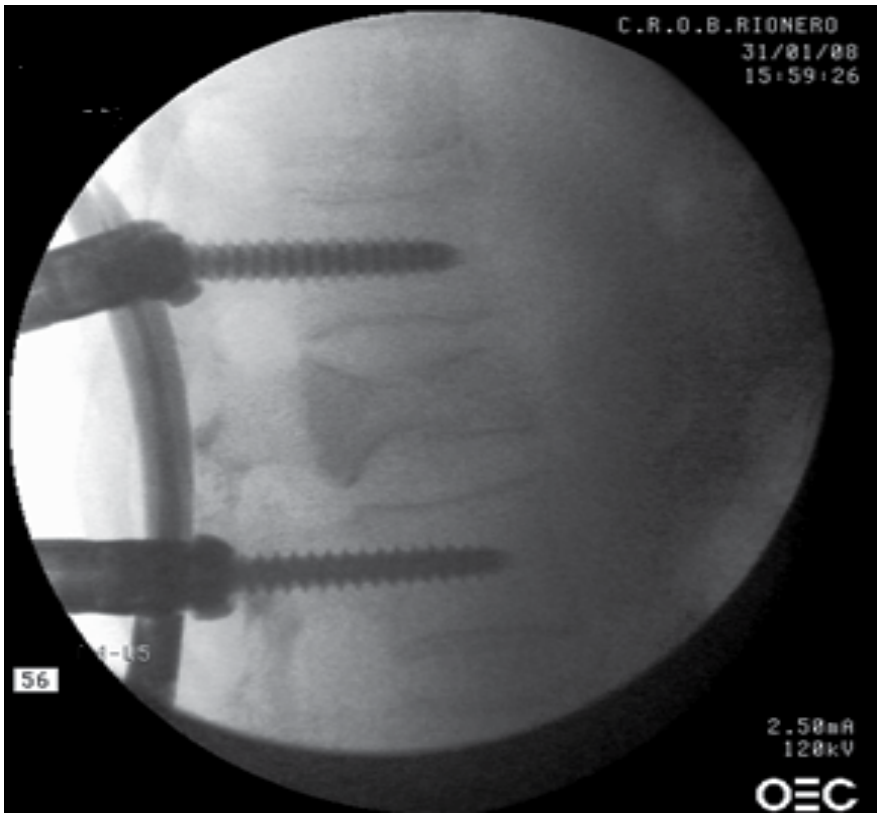

Fig. 7. LL X-ray image of percutaneous screw assisted spinal stabilization device

The immediate analgesic effect is due to cement injection into the fracture, while the long-term effect is guaranteed by stabilization or correction of spinal deformity, which guarantees not only the restoration of proper biomechanics but also the reduction in fracture risk of other vertebrae. The improvement of quality of life is significant, allowing more motor activity of the patient, which in turn leads to better preservation of bone mass and thus fracture risk containment, not only of the spine.

6. Controversies

Buchbinder et al. in 2009 performed a multicenter, randomized, double-blind, placebo-controlled trial in which participants with painful osteoporotic vertebral fractures not older than one year and unhealed, were randomly assigned to undergo vertebroplasty or a sham procedure. Outcomes were assessed at 1 week and at 1, 3, and 6 months; the primary outcome was pain evaluation at 3 months. They found no beneficial effect of vertebroplasty as compared with a sham procedure in patients at 1 week or at 1, 3, or 6 months after treatment.

Kallmes et al. in 2009 in a multicenter trial, randomly assigned 131 patients with painful osteoporotic vertebral compression fractures to undergo either vertebroplasty or a simulated procedure without cement; patients were allowed to cross over to the other study group after 1 month. For those receiving the sham procedure, 42% opted to receive VP at three months, compared with 12% for the other arm. The two groups did not differ significantly on Roland–Morris Disability Questionnaire (RDQ) or average pain intensity at 1 month, but there was a trend toward a higher rate of clinically meaningful improvement in pain in the vertebroplasty group . The authors found that improvements in pain and pain-related disability associated with vertebral fractures in patients treated with vertebroplasty were similar to the improvements of control group. Anyway the higher rate of cross-over could reflect dissatisfaction with the sham procedure compared with PV, or possibly flaws in the blinding of the sham procedure such that patients were able to "guess" which intervention underwent. Clark et al. and Baerlocher et al. in 2009 criticized the previous study underlying that a more appropriate selection criterion would have included patients with uncontrolled pain for less than 6 weeks as the number of patients with pain for less than 6 weeks was too small for a subgroup analysis. Moreover the study of Buchbinder had a target enrollment of 200 patients, but only 78 were enrolled over 4 years, substantially limiting statistical power. More criticism evidenced that in the study, described as multicenter trial, two of the four hospitals withdrew early from the study, after enrolling five patients each; 68% of the procedures were performed in one hospital by one radiologist; respectively 64% and 70% of eligible patients declined to participate in trials reported by Buchbinder and Kallmes raising further concerns regarding patient selection. Both trials did not examine the role of VP in non osteoporotic vertebral fractures or in the inpatient setting (Weinstein, 2009) Recently a multicenter study, the so called VERTOS II, randomized over 200 patients with a vertebral compression fracture and pain of less than 6 weeks duration to conservative treatment or VP; participants and physicians as well as outcome assessors were not blinded. Sham procedure was not performed. Authors found a statistically significant reduction in pain in the VP arm after one month and one year (Clazen et al., 2010). Rousing et al. reported a 12-month follow-up from an open-label, randomized study including 50 patients with a vertebral fracture less than 8 weeks comparing VP with conservative management. They observed an immediate and significant pain relief following VP. One month after hospital discharge, patients undergone VP, had a statistically significantly reduction in pain compared with the ones in conservative therapy arm. However, no difference in pain scores have been observed between groups after 3 and 12 months. They suggested that the role of VP may therefore be considered as a short-term method of pain control in those who fail conservative treatment or for those whom conservative treatment and the accompanying immobilization carry serious risks (Rousing et al., 2010).

7. Conclusions

Long-term effectiveness and complication data from VP or KP are currently lacking. Performing a true blinded randomized-controlled trial between conservative therapy and invasive techniques is impossible. It is the authors' opinion that for patients who are failing conservative treatment or are at increased risk from prolonged bed rest, (i.e. older patients or patients with COPD), augmentation techniques could offer a good pain relief in comparison to conservative treatment, even if no durable long-term benefit has been yet demonstrated. On the other side patients with pain of greater than three months duration

are less likely to benefit from these techniques. Patients need to be carefully screened by history, examination, and imaging prior to the procedure, so to identify the subgroup of patients who may really get benefit from these vertebral augmentation procedures. VP and KP remain an important intervention for the treatment of those patients hospitalized due to severe pain following osteoporotic-induced vertebral fracture.

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Part 7

Research and New Challenges in Osteoporosis

33

Osteoporosis: A Look at the Future

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1. Introduction

Osteoporosis – the commonest age-related skeletal chronic disorder– is characterized by loss of bone mass, alterations of bone micro-architecture, and increased fracture risk. It has, however, received much less attention than most chronic diseases. Osteoporotic fractures are expensive to treat, and cause significant mortality, morbidity, and loss of independence in an ever broader population of patients. As we have said, osteoporosis is a disease in which the mineral density of the bone (BMD) is reduced, its microarchitecture disrupted, and the expression profile of non-collagenous proteins altered. All these factors predispose bones to fractures, particularly the hip, spine and wrist, and are a major cause of disability, severe back pain and deformity. The World Health Organization estimates that approximately 70 million people worldwide have osteoporosis (Penrod J. et al., 2008). The total cost of osteoporosis is difficult to calculate because it includes in-patient and out-patient medical care, loss of working days, chronic nursing-home costs, and medication. The direct costs of osteoporosis arise mainly from the management of patients with hip fractures. Hip fractures also account for 40% of all deaths from trauma in patients over 75, with 68% of all patients not returning to their former level of activity following an osteoporotic hip fractures. The annual worldwide incidence of hip fracture is 1.5 million, a number projected to grow to 2.6 million by 2025 and to 4.5 million by 2050 (Penrod J. et al., 2008). For these reasons, it is necessary to pay a special attention to these health problems which disturb the quality of life.

1.1 Evidence for genetic variation influencing fractures

Both disease prevention and innovation in therapy are critically dependent on identifying the factors that predispose to the development of osteoporosis (Marini & Brandi, 2010). Several studies that have investigated the influence of genetic factors on the development of osteoporosis have found a weaker contribution than to peak bone mass where up to 80% of

the variance can be explained by genetics alone in both sexes (Ralston & de Crombrugghe, 2006).

High-throughput technologies facilitate the identification of genetic, genomic, proteomic, and metabolomic markers of osteoporosis risk that may find a place in clinical prediction algorithms. The operation of intricate networks of genes, environmental factors, and gene-by-environmental interactions further complicate our understanding of the genetic components of the osteoporosis. Until recently, single nucleotide polymorphisms (SNPs) were thought to be the predominant form of genomic variation and to account for phenotypic variation in patients with osteoporotic disorders and those without. However, with the advent and application of array-based comparative genomic hybridization (aCGH), which allows analysis of the genome with a significantly higher resolution than previously possible, scientists have demonstrated that humans are much more genetically variable than previously thought. In two different publications in 2004 (Iafrate et al., 2004; Sebat et al., 2004) hundreds of genomic regions that varied significantly with respect to the number of copies (CNVs) have been reported. Since then, older observations (Iafrate et al., 2004; Sebat et al., 2004) have been replicated and expanded (Conrad et al., 2006; deVries et al., 2005; Repping et al., 2006; Schoumans et al., 2005; Sharp et al., 2005; Tuzun et al., 2005). CNVs vary greatly in size, with insertions or deletions ranging from below 1 kb to several Mb in length (Feuk et al., 2006). As with other types of genetic variation, some gene CNVs have been associated with susceptibility or resistance to disease (Aitman et al., 2006; Cappuzzo et al., 2005; Feuk et al., 2006; Gonzalez et al., 2005; Redon et al., 2006; Sebat et al., 2007; Deng et al., 2010). In humans, CNVs encompass more DNA than SNPs and may be responsible for a substantial part of human phenotypic variability and disease susceptibility (Freeman et al., 2006; Redon et al., 2006). In spite of revolutionary technologies, the major genes determining the bone density and the fracture risk in humans remain uncertain. Approximately 150 candidate genes that might influence the BMD have been identified (Richards et al., 2009; Zhang et al., 2010). Confirmation analyses have revealed that only 30 of these SNPs are somehow connected with the development of osteoporosis.

Preliminary Pathway analysis (Molecular INTeraction database) of the differentially regulated genes/proteins in patients with osteoporosis (Richards et al., 2009) has revealed significance for the highlighted nodes (Mothers against decapentaplegic homolog (SMAD) 2, 3, 4 and 7, Tumor necrosis factor superfamily (TNFRSF), Integrin beta-3 (ITGB3), Bone morphogenetic proteins (BMPs), Transforming growth factor beta-receptors (TGFBRs), Calmodulin 3 (CALM3), TANK protein, Cystic fibrosis transmembrane conductance regulator (CFTR), Pro-neuropeptide Y (NPY)), suggesting that these pathways might play a concomitant role in the pathogenesis of osteoporosis (Fig. 1). New findings notwithstanding, we have to remember that the formation of bone and its repair is a complex process including skeletal patterning, remodeling, and bone growth. The rate of bone formation is dependent on the commitment and replication of several cell types, including osteoprogenitor cells. Their differentiation into functional osteoblasts and the life span of mature osteoblasts is very important. Although a few signaling pathways and patterns of gene expression have been identified in the process of osteoblast differentiation, the exact molecular mechanisms are poorly understood.

A recent genome wide association study (GWAS) has identified approximately 9000 CNVs in patients with lower BMD at spine, hip, and femoral neck (Deng et al., 2010). This study showed that only one low copy number variant of VPS13B gene had some possible

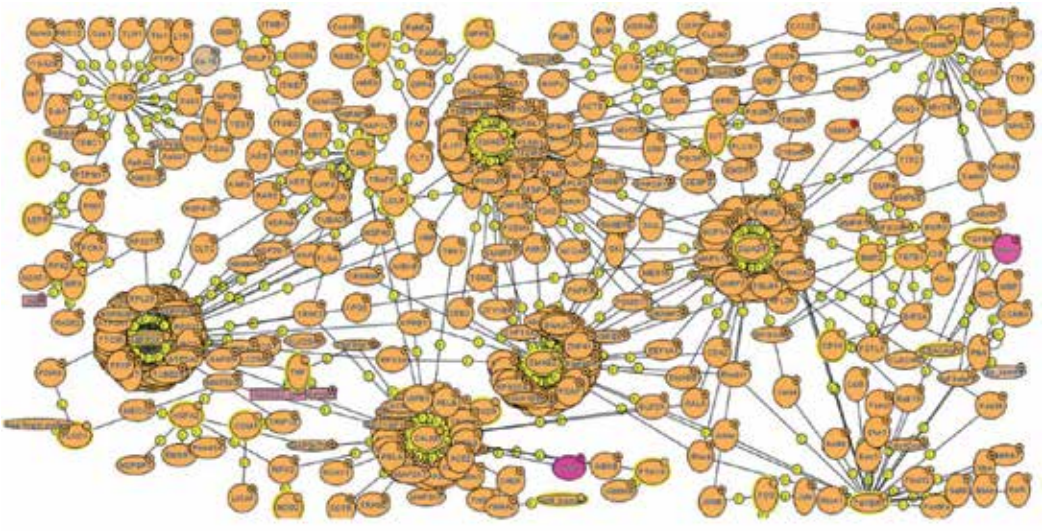

Fig. 1. Overview of signal-transduction pathways associated with candidate genes underlying susceptibility to osteoporosis.

protective role associated with stronger bones and reducing the risk of osteoporotic fractures. The extent to which genes cause alteration in combination with different risk factors for fracture might explain the incidence of fractures in the population, and will depend on strength of association between the risk factor and fracture risk, and on proportion of the population at different levels of allele frequencies (Hopper, 2000). Therefore, although a variation in a risk factor may be strongly genetically determined, it may have little consequence for the disease in terms of explaining different patient's clinical outcomes.

2. State-of-the-art

Bone fracture healing is a complicated, multistage process, influenced by cells events and regulated by local and systematic factors. Recent data concerning the regulatory factors correlated with fracture healing suggest that several chemical compounds can be used to stimulate bone growth, and enhance callus formation and maturation. The pharmacological approach towards osteoporosis prevention aims to increase the bone mass by decreasing of osteoclastic bone resorption with the aid of estrogens, bisphosphonates, calcitonin, calcium plus, cholecalciferol, calcitriol and selective estrogen receptor modulators (Grabo & Longyhore, 2008).

Moving away from these conventional approaches, investigators have recently used lovastatin loaded nanoparticles (Garrett et al., 2007) or Ossein – Hydroxyapatit compounds (OHC) for osteoporosis treatment. One of the most important pleiotropic actions of statins is their effect on bone metabolism (Horiuchi & Maeda, 2006). Statins form a class of hypolipidemic agents, known as 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitors, commonly used as therapy to lower the cholesterol levels in people with/or at risk of cardiovascular disease. Statins exhibit a pleiotropic effect in preclinical models, accelerating fracture healing and increasing angiogenesis when the fracture area is

systemically or locally treated. OHCs also have osteogenic and chondrogenic properties *in vitro* and accelerate fracture healing *in vivo* (Annefeld et al., 1986).

Some patients respond poorly to these therapies, including the integration of implants such as hip replacements. They develop post-surgical complications, caused mainly by weakening of the implant-bone bond due to bone resorption at the interface. This is caused by necrosis of the surrounding host tissue due to surgery, heat released during cement polymerization and micromotion caused by poor fixation. To improve the integration of implants with weak osteoporotic bone, fixation augmentation techniques have been recently proposed such as superficial coatings of polymethylmethacrylate, calcium phosphate ceramics or hydroxyapatite (HA) onto the metallic surfaces (Annefeld et al., 1986; Moroni et al., 2001; Moroni et al., 2005a; Moroni et al., 2005b). Metal surfaces coated with nanohydroxyapatite or biomimetic calcium phosphate (CaP) have been used to improve the fracture fixation stability, which has been proposed as a strategy for porous bones. However, the conventional method for CaP-coating requires high temperature treatment, which prevents the addition of bioactive pharmaceuticals. Post-treatment loading with drugs generally results in their uncontrolled, high kinetic release. A biomimetic nano-CaP coating technology which retains all the features of plasma spraying, including controllable porosity and 3D morphology was recently developed. This technology results in the deposition of amorphous CaP coprecipitated with bioactive substances through the full thickness of the coating, allowing gradual release. The influence of resorbable CaP particles and paste on bone healing was investigated by Bloemers et al. (Bloemers et al., 2003). Twelve weeks after the defect reconstruction, radiological, biomechanical, and histological analyses were performed. Biomechanical tests showed a significantly higher torsional stiffness for the resorbable CaP paste group when compared with the autologous bone.

Several other investigations (de Melo et al., 2006; Niemeyer et al., 2010) published recently utilized the transplantation of xenogenic human and autologous ovine mesenchymal progenitor cells in an ovine critical-size defect or study whether blood-derived endothelial progenitor cells promote the bone regeneration once transplanted into an ovine and tibial defect in animal models (Rozen et al., 2009). Both studies showed significant bone regenerative processes, but the overall results are inconclusive.

Nanoscale materials currently investigated for bone tissue engineering applications can be placed in the following categories: ceramics, metals, and composites. Each type has distinct properties that can be advantageous for specific bone repairing applications. For example, HA, an inorganic compound of bone can be made synthetically. Ceramics are not mechanically tough enough to be used in bulk for large scale bone fractures. However, for a long time they have found applications as bioactive coatings, owing to their ionic bonding mechanisms that are favorable for osteoblast functions (Hench, 1982; Hench, 2004). Unlike ceramic materials, metals are not present in the body as bulk materials. However, because of their mechanical strength and relative inactivity with biological substances, metals and alloys (Ti, Ti_6Al_4V, CoCrMo, etc.) have been the materials of choice for large bone fractures. Composites of the above mentioned materials can be synthesized to provide a wide range of material properties and also to increase the bone implant performance (Nikolovski & Mooney, 2000). It was found that detonation-generated nanodiamond (DND) inclusions can stimulate the biological performance of the composite layer (Pramatarova et al., 2007).

Although novel bioactive and organic materials with good bioavailability and osteogenic activity such as new nano CaP and sulfate cements, bioactive glass and polymers, have been

developed in last years, their application in the treatment of osteoporotic bones is limited because of their weak angiogenic properties.

3. New concepts and strategies for the treatment of osteoporosis

A significant number of osteoporotic bone fractures are treated with implants which are generally fixed with bone cement. However, this fixation requires good quality cancellous bone, generally absent in the osteoporotic population. The incidence of implant loosening and revision surgery is high when osteoporosis is present. For years, attempts have been made to use tissue engineering to develop functional substitutes for damaged or diseased tissues through complex constructs with living cells, bioactive molecules and three-dimensional scaffolds, which can support cell attachment, proliferation and differentiation. By integrating of nanotechnology with cellular and molecular biology we aimed to develop injectable, bioresorbable polymers, in acellular and autologous cell-seeded forms, to enhance the bone fracture fixation and healing, and to promote regeneration of the natural tissue.
New strategies involve:

- Preparation of injectable scaffolds
  - Incorporation of angiogenic/osteogenic factors within the scaffolds (if necessary)
  - Homogeneous distribution within a cavity of any shape
  - Easy integration of biologically active substances and nanomaterials for improvement of the pro-angiogenetic properties of bone macroporous scaffolds
  - The ability to deliver progenitor cells as autologous transplant
  - Manipulation of cell function
  - Avoidance of foreign body reactions
  - Transplant assimilation and remodelling
- Preparation of implants with tailored properties
  - Enhancing minimally invasive application and securing better apposition between bone tissue and material
  - Deposition of angiogenic extracellular matrixes on the surfaces
  - Preparation of new implants with increased biocompatibility by specific nanocoating technology.

Strategies that allow efficient scaffold injectability are critical for the realization of successful treatment for osteoporosis with minimally invasive surgery.

4. Methods

4.1 Synthesis of PLGA-PEG-PLGA triblock copolymers

The synthesis of block copolymers with a general structure PLGA-PEG-PLGA was accomplished as follows: A mixture of polyethylene glycol (PEG), lactide and glycolide with a predetermined molar ratio (Table 1) was placed into a polymerization flask and dried by azeotropic distillation using anhydrous toluene as an entraining agent. After heating at 80 °C under stirring, 0.5 mL of 0.06 M $Sn(Oct)_2$ was rapidly injected through a septum into the polymerization mixture. Then the reaction temperature was elevated to 140 °C and maintained constant for 24 hours. After that, the polymerization flask was cooled down to room temperature and the reaction mixture was dissolved in a small amount of CH_2Cl_2. The copolymers were collected by precipitation in cooled diethyl ether and dried at 40 °C in vacuum for few minutes. The obtained copolymers were dissolved in cold water and then

heated to 80 ºC to precipitate, and remove the water-soluble impurities and unreacted polyethylene oxide. The purification process was repeated three times.

Sample	Lactide, g	Glycolide, g	PEG, g	Yield,%
$PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$	8.352	1.665	5.000	97
$PLGA_{900}$-PEG_{1000}-$PLGA_{900}$	7.500	1.499	5.000	93
$PLGA_{800}$-PEG_{1000}-$PLGA_{800}$	6.665	1.330	5.000	98
$PLGA_{700}$-PEG_{1000}-$PLGA_{700}$	5.832	1.165	5.000	87

Table 1. The starting monomer and oligomer amounts for the synthesized block copolymers and their yields.

The polymers were characterized by ^1H NMR (250 MHz) spectroscopy recorded with a Bruker Avance DRX 250 apparatus, Fourier transforms infrared (FTIR) spectroscopy, and Size exclusion chromatography (GPC). GPC was performed on Waters chromatographic system, equipped with a double detection- differential refractometer M410 and M484 UV detector. Data collection and processing were done using Clarity software. The analyses were performed on Ultrastyragel Linear, Styragel 100 Å, and Styragel 500 Å columns (Waters) calibrated with polyethylene oxide (PEO) standards. Tetrahydrofuran was used as mobile phase with a flow rate of 1.0 ml/min at 45 °C. FTIR spectra (KBr pallets) were recorded on Bruker-Vector 22 FTIR spectrometer at a resolution of 1-2 cm^{-1} accumulating 50 scans.

4.2 Nanocrystalline diamonds production and characterization

Shock-wave synthesis was used to produce nanocrystalline diamonds (NCDs) by explosive conversion of a trinitrotoluene/hexogene mixture with negative oxygen balance (Ivanova et al., 2011). A water-cooled combustion chamber with 3.0 m^3 volume was applied. A mixture of diamond blends containing 85% NCDs was obtained. The NCDs were purified by oxidative removal of the non-diamond carbon using a mixture of $K_2Cr_2O_7$ and H_2SO_4 according to (Tsoncheva et al., 2006). NCDs were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), and energy dispersive X-ray spectroscopy (EDS). The XRD spectra were obtained with a Bruker D2Phaser diffractometer with CuKα radiation in 2 theta range between 30 and 110° with a step of 0.02° and a measuring time of 10 s per point. The size and morphology of NCDs were studied by TEM. The experiments were performed with a Philips EM420 transmission electron microscope with accelerating voltage of 120 kV. The sample was suspended by ultrasonic agitation in ethanol at room temperature and an aliquot of the solution was dropped on a holey carbon film supported on a copper grid. The impurities present in the samples were analyzed with an EDAX 9100/70 attached to the microscope.

4.3 Preparation of polymer hydrogels and its lovastatin and NCD loaded forms

The polymer hydrogels were obtained by dissolving of the PLGA-PEG-PLGA polymers in deionized water at suitable temperatures - below and above the so-called "gelation temperature". Equal amounts of freeze-dried PLGA-PEG-PLGA polymers were dissolved in different amounts of sterile water to prepare samples with desired concentrations (10, 15, 20, and 25% (w/w)). The total solubility of the copolymers was achieved by continuous shaking of

the samples at a temperature of 4.0 °C for 48 h. The gel-sol transition behavior of the block copolymer solutions was investigated following the procedure described by Lee et al. (Lee et al., 2006) using a WB-4MS water bath-thermostat (Biosan) and also was studied by rheological analysis (Yu et al., 2010). The introduction of NCDs in the polymer hydrogels was made by preparation of sterile aqueous NCD suspensions with different concentrations, used in order to get a final concentration of 0.5 mg/ml in the hydrogels. The sterilization of the NCDs was achieved through stepwise washing with 99% and 45% ethanol and autoclaved deionized water. Lovastatin loaded hydrogels were obtained by dissolution of lovastatin (powder) in the already prepared hydrogels. The final concentration of lovastatin was 825 μM.

4.4 *In vitro* degradation of the polymeric hydrogels

The degradation of the polymeric hydrogels (166 μl of 25% (w/w) $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ or $PLGA_{900}$-PEG_{1000}-$PLGA_{900}$) was determined by their incubation in the presence of 5.0 ml EPCs cell culture media (see below) in 24 well plates, in a humidified 5.0% CO_2 incubator at 37 °C for 10, 20, and 30 days. Following the incubation periods, the cell culture media was removed and the remaining hydrogels were washed with distilled H_2O several times and dried under vacuum up to a constant weight. The obtained dried polymer residuals were dissolved in $CDCl_3$ and characterized by ^1H NMR spectroscopy. The hydrogels degradation was also followed by phase contrast microscopy (Carl Zeiss Teraval 3) equipped with a DCM 300 digital camera (Hangzhou Huaxin IC Technology Inc., China).

4.5 Preparation and surface modification of nanodiamond films for implants functionalization

The ultrananocrystalline diamond (UNCD) coatings discussed in this chapter were grown by microwave plasma chemical vapour deposition (MWCVD) from gas mixtures containing 17% CH_4 in N_2. The substrate temperature was kept 600 °C, the working pressure 2.2 kPa, the microwave power 800 W, the deposition time 360 min. Detailed description of the MWCVD set-up is given in (Popov et al., 2006b). Monocrystalline silicon wafers were used as substrates which were ultrasonically pretreated in a suspension of diamond powders with different fractions in n-pentane prior the deposition in order to enhance the diamond nucleation on the surface. Such coatings were prepared also on a number of other materials of biomedical interest, for example, Ti-alloy used for implants (Kulisch & Popov, 2006).

In order to tailor the surface properties of the UNCD films with respect to their wettability, surface conductivity, etc., they were subjected to a number of surface modification processes as follows: i) H_2 microwave plasma at 400 °C in the deposition set-up (in the following: *HP*); ii) O_2 microwave plasma at room temperature (*OP*); iii) CHF_3 plasma in a 13.56 MHz parallel plate reactor also at room temperature (*FP*); iv) NH_3/N_2 plasma in a 13.56 MHz plasma enhanced chemical vapour deposition set-up at room temperature (*NP*); v) UV/O_3 photochemical treatment at room temperature (*UV*) and vi) chemical treatment with aqua regia (HCl/HNO_3 with a ratio of 3:1) at room temperature (*AR*). Further experimental details can be found in several references (Koch et al., 2011; Kulisch et al., 2010; Popov et al., 2008b).

4.6 Isolation and characterization of endothelial progenitor cells (EPCs)

Endothelial progenitor cells were isolated and cultured with minor modifications of the protocols described in earlier works (Fuchs et al., 2006a; Fuchs et al., 2006b). In brief, mononuclear cells were harvested from human peripheral blood buffy coats using Ficoll

(Sigma-Aldrich) gradient centrifugation and cultured in endothelial cell growth medium-2 (EGM-2 kit; CC-3162, Lonza, Belgium), 5.0% fetal calf serum (FCS; Lonza, Belgium), and 1.0% penicillin/streptomycin, on collagen-coated (BD Europe, Germany) well plates, where 5×10^6 cells per well were seeded. The good colonies of EPCs appeared after 3 to 4 weeks in culture. The EPCs' phenotype characterization was done following the protocol of (Fuchs et al., 2006a).

4.7 Transformation of EPCs to osteoblast

Two days after colony formation, the EPCs were trypsinized to single cells, passed through a 70 μm filter, and plated on 1.0 mm cover slips coated with hydrogels at 25 cells/cm^2 in osteoprogenitor medium (IMEM, Invitrogen) supplemented with 10% fetal bovine serum (Lonza, Belgium), 0.1 mM 2-mercaptoethanol, 2.0 mM Glutamax I, 2.0 mM BMP-2, and 0.2 mM ascorbic acid. The cultures were fed with osteoprogenitor medium every 2-3 days and allowed to differentiate up to 21 days to form mature bone nodules on different substrates. The transformation of endothelial phenotype of EPCs into long-term osteoblast culture growing on hydrogels or UNCD coatings was assessed as described previously (Popov et al., 2008a; Trajkovski et al., 2009) and followed by phase contrast and fluorescent microscopy, real time PCR (qPCR) and Western blot **(**Trajkovski et al., 2009**).** RNA and proteins were isolated with Trisol (Invitrogen, USA) following manufacturer's instruction on Day 1, 7, 14 and 21 after EPCs seeding on hydrogels and nanocomposites. Prior to amplification, the RNA purity and integrity were checked on Nanodrop-1000 (Termo-Scientific) and agarose gel electrophoresis. SYBR Green qPCR analysis was performed on RotorGene-6000 (Corbet) with the primers sequences and conditions as described by Woll et al (Woll et al., 2006; Woll & Bronson, 2006).

4.8 *In vivo* experiments

This study was carried out in accordance with the guidelines of the Medical University Pleven Ethics Committee (N 20/21.12.2010). A total of 24 adult female Wistar rats (220– 250 g, 3 animals per group) were used. Following i. p. injection of 45 mg/kg b.wt. ketamine a sterile 25% (w/w) $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ polymers with or without lovastatin (825 μM) or nanodiamonds (500 μg/ml) were implanted s. c. The animals were sacrificed on day 1 and 30 days later, and the explants were taken for further analysis.

4.9 Statistical analysis

The data were evaluated by analysis of variance (ANOVA) followed by Tukey's post-hoc test. Differences in the results at the level of $p<0.05$ were considered statistically significant. The statistical analysis was carried out using the PASW 18.0 statistical software package (IBM) for Windows.

5. Results

The structure-function properties of the native bone tissue pose strong design criteria to the substrates required to engineer their living counterparts or to be used in endogenous repair. When designing these substrates one should consider the macroscopic (organ level, geometry), the microscopic (cell and tissue level, scaffold architecture), and molecular properties relevant for tissue morphogenesis and function (e.g. hematopoiesis). In addition,

developmental aspects, such as growth, differentiation, migration and maturation should be taken into account, as these may dictate substrate properties, like degradation and porosity, or require additional instrumentation of bioactive factors (Bouten et al., 2011). Such scaffolds with a possible application for *in bone injection* have to be designed and studied very carefully, because the progenitor cells derived from bone marrow may also be homed on to the bioactive substrate.

5.1 Characterization of three-dimensional (3D) scaffolds

The composition of PLGA-PEG-PLGA block copolymers and its average molecular weight (M_n) were determined by ^{1}H NMR spectroscopy (Fig. 2). The broad chemical shift signal at 3.65 ppm marked as **b** is a characteristic of the methylene protons of the ethylene oxide repeating units. The signals observed at 1.58 ppm (signal **a**) CH_3-CH-, 5.2 ppm (signal **d**) CH_3-CH-, and 4.8 ppm (signal **c**) -O-CH_2-C(O)- are assigned to protons of methyl, methine, and methylene groups in lactide and glycolide units, respectively. The integrations (I_a, I_b, I_c, I_d) of the signals observed at 1.58, 3.65, 4.8 and 5.2 ppm assigned above were used for calculation of average molecular weight of all block copolymers following the equations:

$$M_{n\,(NMR)} = [(I_a/3)M_{Lac} + (I_c/2)M_{Gly} + M_{EO}] \times 4I_bDP_{PEG} \quad (1)$$

or

$$M_{n\,(NMR)} = [(I_dM_{Lac} + (I_c/2)M_{Gly} + M_{EO}] \times 4I_bDP_{PEG} \quad (2)$$

The DP_{PEG} is the degree of polymerization of the PEG block and $M_{Lac,}$ M_{Gly} and M_{EO} are the molecular weights, of the lactic, glycolic, and ethylene oxide segments. The calculated values of the average molecular weight of the block copolymers are represented in Table 2.

Sample	*M_n, (Theor.)*	*M_n, (NMR)*	*M_n, (GPC)*	*M_w/M_n, (GPC)*
$PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$	*3000*	*3150±122*	*3300±169*	*1.25*
$PLGA_{900}$-PEG_{1000}-$PLGA_{900}$	*2800*	*2948±151*	*3140±115*	*1.17*
$PLGA_{800}$-PEG_{1000}-$PLGA_{800}$	*2600*	*2873±177*	*2700*	*1.11*
$PLGA_{700}$-PEG_{1000}-$PLGA_{700}$	*2400*	*2664±113*	*2621*	*1.21*

Table 2. Characteristics of the PLGA-PEG-PLGA triblock copolymers. Data are representative from twelve independent syntheses and are expressed as average ± S.D.

The results showed deviations between the theoretically expected and the experimentally calculated molecular masses of all polymers. These deviations could be attributed to the presence of some moisture in the organic substances – monomers and PEG oligomer. The NMR data and those obtained from the GPC analysis reveal similar values for the molecular weight of all polymers. The received dispersity (M_w/M_n) indicates the narrow molecular weight distribution(see Table 2), which is typical for polymers prepared by a "living", controlled polymerization.

The composition of the polymers was also proved by FTIR spectroscopy. The spectra of all polymers show similar bands and signals. Only slight differences in the intensity of the bands attributed to the ester and ether type bonds at 1760 and 1163 cm^{-1} were observed and referred to the differences between the numbers of monomer units included in the polymer chains (data not shown).

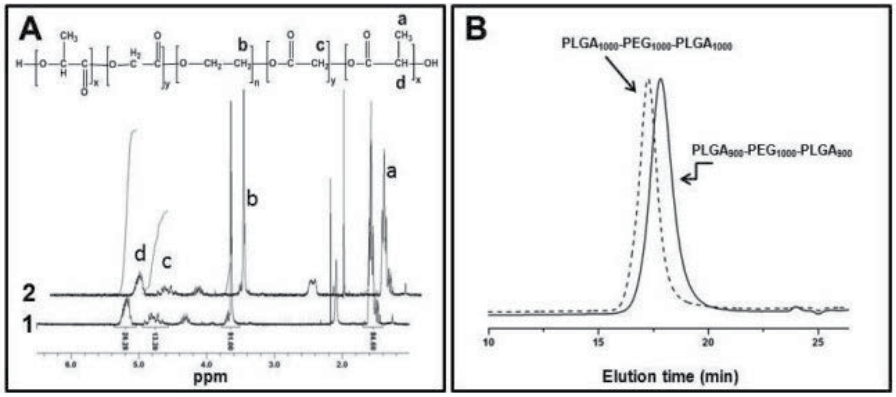

Fig. 2. Chemical characterization of the polymers. A: ^{1}H NMR spectra of $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ (1) and $PLGA_{900}$-PEG_{1000}-$PLGA_{900}$ (2) block copolymers; B: Molecular weight distribution overlay patterns of indicated polymers received by gel-permeation chromatography.

The copolymers with an analogous structure but a different ratio between the hydrophilic (PEG) and hydrophobic (PLGA) blocks have been an object of considerable scientific interest and value. The influence of the hydrophilic/hydrophobic ratio between PEG and PLGA blocks, and of the block length on the sol-gel–sol transition properties of PLGA-PEG-PLGA triblock copolymers has been reported (Lee et al., 2001). The authors have also found that the effects of different intramolecular/micellar behavior are accompanied by drastic changes in the area of gel zone.

5.2 Temperature–induced sol-gel phase transition of PLGA-PEG-PLGA block copolymers

In our work we chose to investigate PLGA-PEG-PLGA copolymers with much lower hydrophobic PLGA content (PEG:PLGA = 1:2 ; 1:1.8). The advantages of such hydrogels and especially their ability to be loaded with bioactive substances, drugs, cells, nanoparticles and etc., prior administration are of particular interests. The phase behavior (reversible sol-gel-sol transitions) of the obtained block copolymers in aqueous media at different temperatures and concentrations are illustrated in Fig. 3. As shown, only $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ and $PLGA_{900}$-PEG_{1000}-$PLGA_{900}$ copolymers possessed the typical gel-sol phase transition behavior upon heating. They have the ability to form temperature dependent micellar aggregates and after further temperature increase form gels due to micelles aggregation and/or packing. In contrast, the other two copolymers showed a vastly different performance. As the longer PLGA chains are present, stronger hydrophobic interactions are detected, leading to an increase in the aggregation and packing between the polymer micelles resulting in the formation of a denser gel state. The gel zone in the phase diagram of $PLGA_{800}$-PEG_{1000}-$PLGA_{800}$ and $PLGA_{700}$-PEG_{1000}-$PLGA_{700}$ copolymers was not found. Nevertheless, we observed significant alterations of their viscous properties – changes from amber viscous to white viscous state. With additional rise of the temperature, direct transition from white viscous to suspension state was also observed. The slight increase in the temperature enhances the thermal motion of the hydrophobic chains in the $PLGA_{800}$-PEG_{1000}-$PLGA_{800}$ and $PLGA_{700}$-PEG_{1000}-$PLGA_{700}$ copolymers, which may lead to the disturbance of the micellar structures and polymer precipitation.

Injectable biodegradable copolymer hydrogels, which exhibit a sol–gel phase transition in response to external stimuli, such as temperature changes, have found several biomedical and pharmaceutical applications, such as drug delivery, cell growth, and tissue engineering (Gao et al., 2010; Kan et al., 2005; Kim et al., 2001; Nguyen & Lee, 2010; Qiao et al., 2006; Zentner et al., 2001). Such polymers are also biocompatible and biodegradable, and they represent an ideal system for treatment, being able to overcome the problem of carrier removal after injection (implantation). Hydrogels sensitive to temperature are useful for both *in vitro* and *in vivo* applications. These applications will strongly depend on hydrogels composition and it is important to administer the sol dispersion upon quick transformation to gel under physiological conditions. The composition, morphology and crystallinity of the block copolymers strongly influence the mechanical properties and rate of degradation under specific conditions. To study the *in vitro* behavior of the $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ and $PLGA_{900}$-PEG_{1000}-$PLGA_{900}$ copolymers we used light microscopy and ^1H NMR spectroscopy.

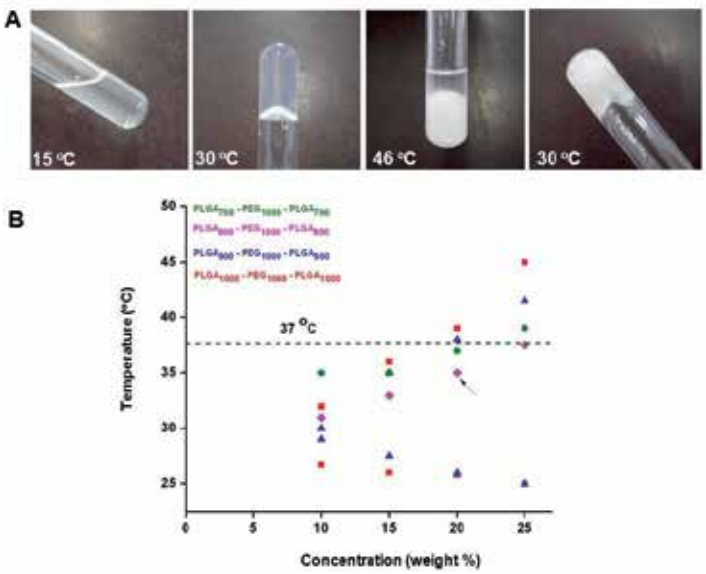

Fig. 3. Sol-gel transitions of $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ block copolymer (A) and phase diagrams of PLGA-PEG-PLGA triblock copolymers (B).

5.3 *In vitro* degradation of the polymeric hydrogels

Polymer degradation is the key process of erosion and plays a crucial role for all polymers studied. The processes involved in the erosion of a degradable polymer are very complicated. They can be summarized as follows: (1) water enters the polymer bulk; (2) water entrance may be accompanied by swelling; (3) chemical polymer degradation appears, leading to the creation of oligomers and monomers.; (4) the degradation changes the microstructure of the hydrogels through pore formation; (5) oligomers and monomers are released; (6) dissolution and diffusion of oligomers and monomers are undergoing; (7) the pH inside the pores can be changed, depending of polymer composition; (8) finally, the released oligomers and monomers lead to the weight loss of the hydrogels.

Fig. 4 reflects the hydrogels morphology after 10 and 30 days in contact with 5.0 ml EPC culture media at 37 °C. The 20 and 25% (w/w) $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ and $PLGA_{900}$-PEG_{1000}-$PLGA_{900}$ copolymer samples were about 1.0 mm thick and 1.0 cm in diameter.

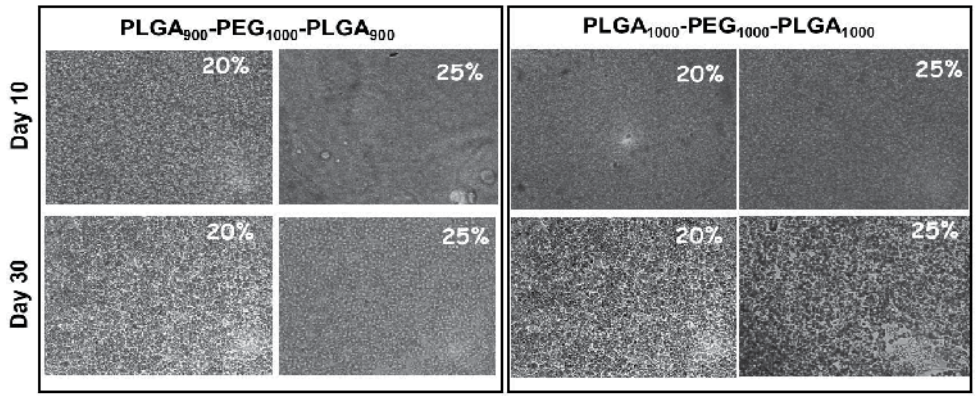

Fig. 4. Optical microscopic images of the polymeric hydrogels during their destruction in cell culture environment. Magnification: x100.

Changes in the weight-average molecular weight (M_W) of PEG and PLGA components in the scaffolds as a function of degradation time are shown in Fig. 5. It can be seen that the M_W of hydrogels decreased with incubation time for all of the scaffolds. There were no significant differences in the degradation rate between 20 and 25% hydrogels. The decrease in molecular weight can be attributed to hydrolysis and macromolecular scission of PLGA (Shih et al., 1996). Monomer release profiles for PLGA have a short induction period. The release rate was higher at early times and declines in a concave manner.

The acidic degradation products of PLGA did not lead to severe decrease in the pH values. It drops from 7.4 (Day 1) to 6.4 (Day 30). To evaluate the degradation of the scaffolds, the medium pH for the specimens was compared to media that was held under the same conditions but did not contain any samples (blank). The EPC's media with a lower pH than the blank one would indicate the release of acidic products from the scaffolds and can lead to faster degradation.

By 24 h the difference between the blank media pH and the pH of the media in contact with hydrogels was negligible and remained so through 1 week. On day 10, the pH of the media incubated with hydrogels began to decrease. This decrease in pH persisted through week 2 and 3, with a maximum deviation from blank pH of - 0.94 ± 0.06 at week 4.

It was noticed that the weight loss was about 39% after 30 days degradation in the cell culture media, however, it was 30% just after 10 days. We used the formulas (1) and (2) mentioned above to calculate the changes in the hydrogels' molecular weight - molar content of the different co-compounds. It should also be kept in mind, that the obtained values are relative and don't represent actual PLGA and PEG content in the hydrogels or overall molecular weight of the polymeric chains, but can be used for the construction of a simple qualitative model describing their destruction. Looking at Fig. 5 we can see significant changes or decreasing in the content of PEG part in hydrogels i. e. decreasing the molecular weight of the corresponding copolymers with the time. Similar indicated changes in the hydrophobicity of the polymeric materials of similar type have also been reported by (Youxin et al., 1994; Zweers et al., 2004).

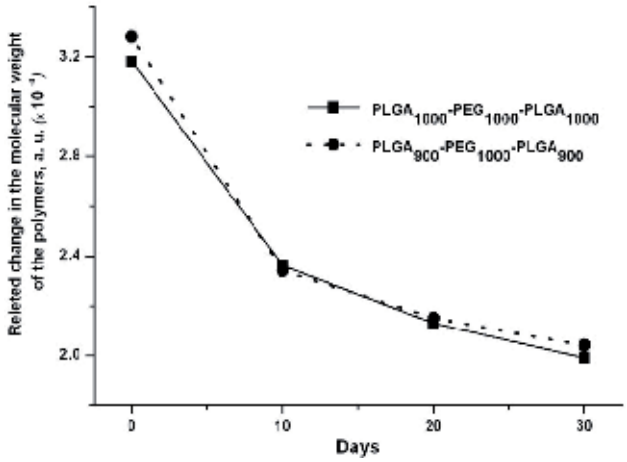

Fig. 5. The relative change in the composition of the block copolymers with the time. Alteration in the molecular weight of PLGA are calculated at a constant PEG content and molecular weight.

The mechanism of erosion/degradation of the polymeric hydrogels can be presented by the general scheme shown in Fig. 6. It can be summarized as follow: (1) the amphiphilic PLGA-PEG-PLGA three-block copolymers form stable micelle solutions; (2) under the influence of thermal fluctuations on the polymeric chains, the micelles are packed into a crystalline-like structure – hydrogel; (3) the H_2O immerges into hydrogels, and the quick swelling happens; (4) when the swelling equilibrium is reached, the chains start to break as a function of time; (5) the presence of water provokes bulk degradation of the hydrogels via a random hydrolytic scission of the ester linkages in the vicinity of PEG; (6) sustained release of water-soluble PEG and PEG's end-capped with short PLGA tails; (7) the hydrophobicity of the remaining hydrogel should increase; (8) as the experiments were conducted in cell culture media with high buffer capacity, the further hydrolysis of glycolic–glycolic and glycolic–lactic ester bonds by lactic and glycolic acids will be slow down.

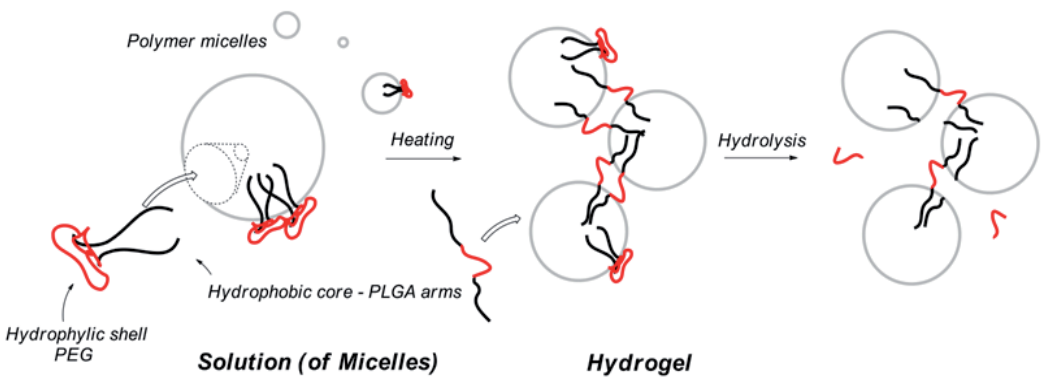

Fig. 6. Schematic representation of hydrogel formation and erosion/degradation.

Sol-gel transition characteristics, including transition temperature and gel window width are the critical parameters which should be taken into consideration in designing 3D

scaffold biomaterials. For *in vitro* and *in vivo* application is also necessary to know their behavior in surrounding environments. To follow the biochemical and molecular biological response of the composites prepared by us we investigated the possibility these polymers to be prepared as a cell loaded form.

5.4 Scaffold colonization and seeding efficiency

The EPCs' attachment and differentiation were performed on sample scaffolds prepared with or without NCDs and lovastatin to evaluate the cell biocompatibility. The experiments suggested that on day 1 of culturing low number of cells was attached to all scaffolds studied. The loading efficiency reached 73.1 ± 5.3% after 3 days on PLGA-PEG-PLGA + NCDs and composites with NCDs and lovastatin (89.6 ± 5.7%) and only 11.4 ± 1.5% for the PLGA-PEG-PLGA + lovastatin. A qualitative analysis of cell adhesion on the scaffolds was carried out by phase contrast microscopy up to 21 days. This study (Fig. 7) demonstrated that the cells were distributed unequally throughout the surface structure with different morphology at the beginning. The results presented in Fig. 7 also confirmed the infiltration and migration of cells deep into the 3D porous network on scaffolds containing NCDs or NCDs + lovastatin only. Cells with distinct rounded nuclei were observed throughout the scaffold, further suggesting normal cell growth on these composites. The results about EPCs adhesion on PLGA-PEG-PLGA scaffolds can found in (Trajkovski et al., 2009; Ivanova et al., 2011).

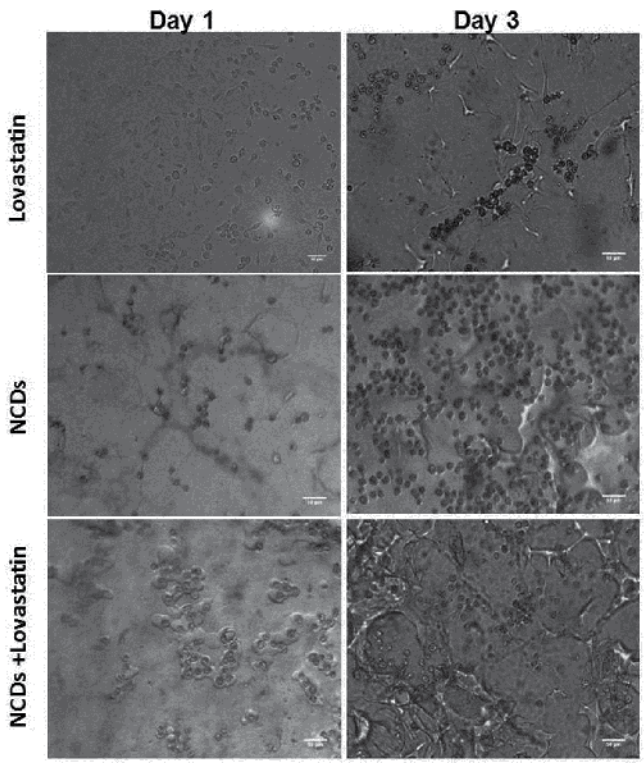

Fig. 7. Endothelial progenitor cells growing on different type composites based on 25% (w/w) $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ hydrogel, containing lovastatin and/or nanocrystalline diamonds (NCDs). Bars 50 μm.

Next we have examined the release of fibronectin (FN) during the EPCs differentiation to osteoblast. Fluorescent photomicrographs of FN stained cells are shown on Fig. 8. As it can be seen the cell response is heterogeneous and FN distributes in a diffuse, punctate pattern of staining throughout the whole surface. The obtained results can be summarized as follows: (1) FN release was detected on day 7 and this was connected with its adsorption to the surfaces; (2) During the process of EPCs differentiation, the release of FN was favored by the hydrogels + NCDs (day 12, 15, and 21). These observations confirmed the hypothesis suggesting that FN is adsorbed preferentially on hydrophobic surfaces (Nordahl et al., 1995), such as the surface of the hydrogels + NCD undergoing erosion. It has also been suggested that FN plays a unique role during the differentiation of osteoblast cultures connected with the formation of mineralized nodules *in vitro* (Robey, 1996). We have reported results confirming this observation (Ivanova et al., 2011).

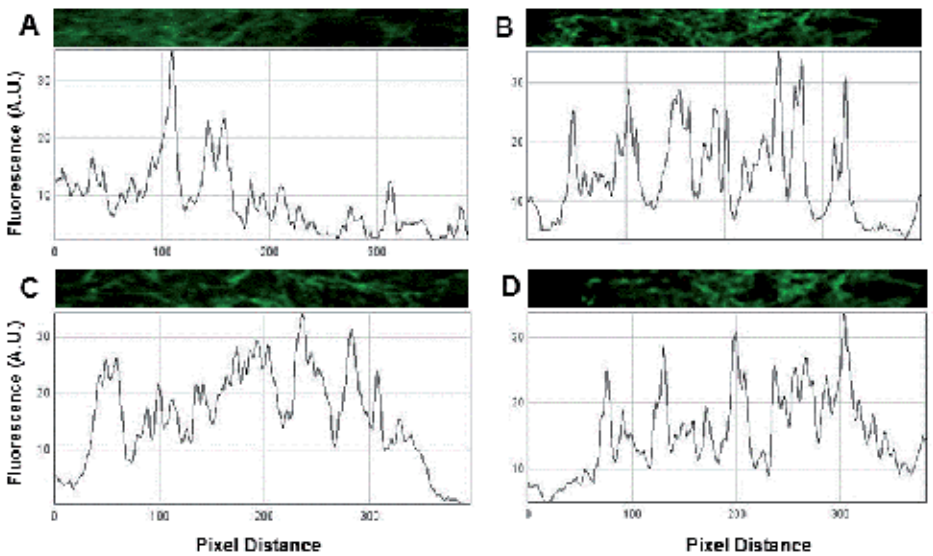

Fig. 8. Composite induced changes in distribution of fibronectin. Hydrogels were based on 25% $_{(w/w)}$ $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ (A); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin (B); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ +nanocrystalline diamonds (C); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin + nanocrystalline diamonds (D). The tracings immediately under each panel are scans of the pixelated fluorescence intensity of fibronectin integrated vertically and scanned horizontally across the magnified field.

All data obtained by us indicated that not only the PLGA-PEG-PLGA scaffolds are biocompatible with the EPCs as reported (Trajkovski et al., 2009; Ivanova et al., 2011), but composites with NCDs actually promote their growth and transformation. The processes of EPCs differentiation can influence the expression of several important genes. We further followed these changes by quantitative real time PCR analyses.

5.5 qPCR analysis of mRNA derived from endothelial progenitors cells undergoing transformation to osteoblasts

The expression of four osteogenic markers *Twist1, Runx2, Osterix,* and *Bglap1*, and the endothelial one - platelet endothelial cell adhesion molecule (*PECAM1)*, was analyzed at

various time points throughout the transformation of the EPCs culture to osteoblasts. The results shown on Fig. 9 revealed a gene expression pattern characteristic of the osteoblast differentiation. The expression of *Twist1 and Osterix* was highly upregulated by day 14 and persisted to day 21 without significant differences between scaffolds studied. *Runx2* and *Bgalp1* expression was continuously increasing during the differentiation and it was found to be significantly higher (Day 7: p=0.034; Day14: p=0.011; Day 21: p=0.07) in EPCs growing on composite scaffolds containing NCDs in comparison with hydrogels ± lovastatin. The PECAM-1 was expressed at the beginning of the differentiation process, but decreased on days 7 and 21. Incorporation of the lovastatin in hydrogels increased the expression of PECAM-1 at day 7 about five times.

In comparison to the hydrogels ± lovastatin, the scaffolds ± NCDs leaded to upregulation of the osteoblasts associated markers at two time points of investigation (Day 7 and 21). In accordance with these observations the total protein amount of Runx2, Osteocalcin and Collagen-α1 was also high (Trajkovski et al., 2009). *Runx2 mRNA* and protein amount were found to increase continuously. Their expression is also critical for mature osteoblast function. RUNX2 binding sites were identified in the osteoblast specific genes encoding osteocalcin, bone sialoprotein, and osteopontin, as well as type I collagen (Ducy et al., 1997). The detected earlier expression of *Runx2 mRNA* might positively influence the latter expression of osteocalcin and collagen-α1 observed by us. The registered increase in *PECAM1 mRNA* demonstrated at Day 1 and 7 also showed the ability of EPCs to switch the differentiation process under specific conditions and confirmed that the lovastatin can stimulate the PECAM-1 expression in endothelial cells. The gene expression data provide strong evidence for osteogenic transformation of the EPCs to osteoblasts on all scaffolds tested.

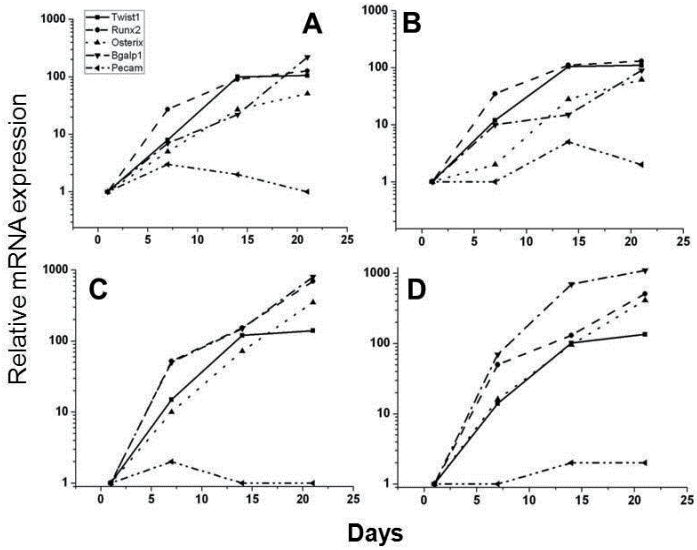

Fig. 9. Quantitative PCR analysis of endothelial progenitor cells (EPCs) undergoing differentiation to osteoblasts. EPCs growing on 25% $_{(w/w)}$ $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ (A); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin (B); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ +nanocrystalline diamonds (C); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin + nanocrystalline diamonds (D). Each assay was run in triplicate at three different template concentrations. Relative mRNA expression is normalized to ribosomal protein L7 (Rpl7), displayed relative to Day 1, and presented as a common log plot.

The tissue engineering is based on the method of cell seeding on scaffolds that play a role as a matrix to guide cell growth and to assist the formation of functional new tissues. Scaffolds grant this process by promoting an appropriate surface and sufficient spaces to favor the cell attachment, migration, proliferation and special differentiation in three dimensional ways. The design of the scaffold is the most important because it influences the above mentioned processes. There are many worldwide accepted criteria for ideal scaffolds with application in tissue engineering and the critical ones include the main material, mechanical properties, 3D architecture, surface morphology and chemistry, as well as the scaffold environment before and after the degradation process (Wei G. & Ma P.X., 2007). Other important properties are: (1) to be biocompatible – non-immunogenic and (2) non-toxic for the living cells and tissue. To test the biocompatibility of the scaffolds prepared by us we have conducted the *in vivo* experiments.

5.6 *In vivo* mass decrease of polymer hydrogels

The 3D-scaffolds loaded with lovastatin and/or nanodiamods and injected in rats provide an evidence for biocompatibility and degradability. To examine time-dependent *in vivo* mass loss of polymer hydrogels, we prepared sterile 25% (w/w) $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ ± NCDs ± lovastatin and injected them into the rats, subcutaneously. Injectable hydrogels for biomedical applications should be degraded or eliminated from the body after accomplishment of their role. Fig. 10 shows *in situ* gel formation of a polymer solution and separated gels explanted at 2 time points. All polymer solutions transformed to hydrogels, and the hydrogels showed time-dependent mass decrease behaviors.

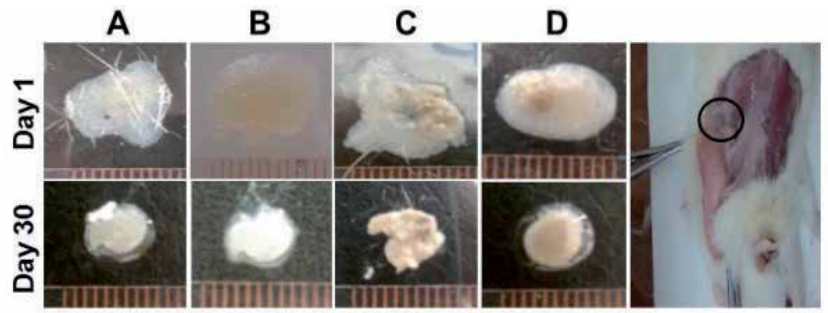

Fig. 10. *In vivo* time-dependent mass decrease of polymer hydrogels. Photographs of separated polymer explants and *in situ* gelation of the polymer solutions from s.c. injected rats. 25% $_{(w/w)}$ $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ (A); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin (B); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ +nanocrystalline diamonds (C); $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin + nanocrystalline diamonds (D).

The hydrogels containing $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ alone showed the fastest mass decrease, but they still remained detectable within 4 weeks. The mass of the polymer hydrogels was decreased in the order of $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + NCDs ≅ $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin + NCDs > $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin > $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$. After 4 weeks, the remained masses of hydrogels were 59%, 58%, 48 and 23%, respectively. We also noticed that around 3 of the $PLGA_{1000}$-PEG_{1000}-$PLGA_{1000}$ + lovastatin loaded hydrogels less capillaries were present, which can be attributed to the negative effect of lovastatin on endothelial cell proliferation and angiogenesis. It may also be connected with the difference of

environment, especially water content. There was no adverse effect during the *in vivo* tests. The observed results suggest that polymeric solutions ± NCDs and ± lovastatin can be used as injectable and biodegradable hydrogels and the degradation rate of the composites can be controlled by adjusting substituent compositions.
Since the scaffolds with NCDs showed very good biocompatibility the next step was to test the opportunity for preparation of new, bio-enhanced UNCD containing films with potential application for implant preparation.

5.7 ND film modifications and the role of implant functionalization

The UNCD films deposited under the conditions described above have been comprehensively characterized with respect to their crystallinity, composition, topography and bonding structure (Popov et al., 2003; Popov et al., 2004; Popov C. & Kulisch W., 2003). The X-ray diffraction and selected area electron diffraction (Fig. 11A) revealed patterns characteristic for diamond phase. Furthermore, the size of the diamond crystallites was determined from the XRD peaks to be on the order of 3-5 nm. These nanocrystallites are embedded in an amorphous carbon matrix with a grain boundary width of 1.0-1.5 nm, as shown by TEM (Popov et al., 2004). The ratio of the volume fractions of the two phases-crystalline and amorphous - estimated from the density of the coatings and from the total crystallinity is close to unity (Popov et al., 2003). Investigations of the UNCD films with Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), electron energy loss spectroscopy (EELS, Fig. 11B) and Auger electron spectroscopy (AES) showed the presence of sp^2-bonded carbon atoms (up to 15 at%) localized in the amorphous matrix (Popov et al., 2003; Popov et al., 2004). Although no H_2 was added to the precursor gas mixture, the UNCD films contain about 9-10 at% H in the bulk, as revealed by elastic recoil detection (ERD) analysis, originating from the CH_4 molecules. Fourier transform infrared spectroscopy showed that hydrogen is bonded predominantly in sp^3-CH_x groups (Popov & Kulisch, 2003).

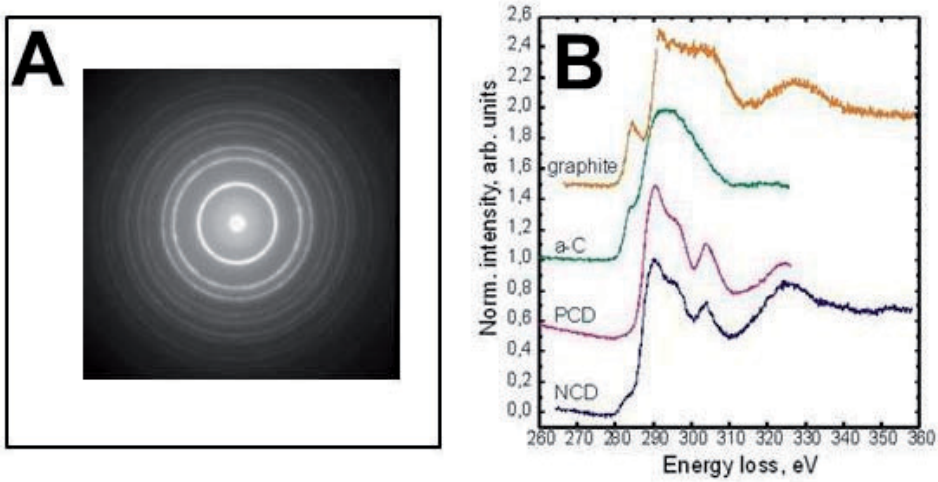

Fig. 11. Physico-chemical characterization of the UNCD films. (A) Selected area electron diffraction of UNCD film; (B) Electron energy loss spectra of different carbon materials (Popov et al., 2004; Popov et al., 2011).

Atomic force microscopy (AFM) studies showed for all investigated samples the characteristic topography of UNCD films, composed of structures with diameters of several hundred nanometers, which themselves possess a substructure (Fig. 12A). The root mean square surface roughness values lie in the narrow range of 10 – 14 nm (Kulisch et al., 2011). The nanostructured surface can expect to enhance the attachment of cells on it. The surface composition of the as-grown UNCD films (*AG* in the following) was investigated by XPS. The results showed that the as-grown surfaces are very clean, with oxygen and nitrogen concentrations of about 2.0 and 1.0 at%, respectively. Nuclear reaction analysis (NRA) revealed the depth profiles of hydrogen concentration in the UNCD films and indicated surface H concentration of 12-14 at% (Fig. 12B)(Kulisch et al., 2008).

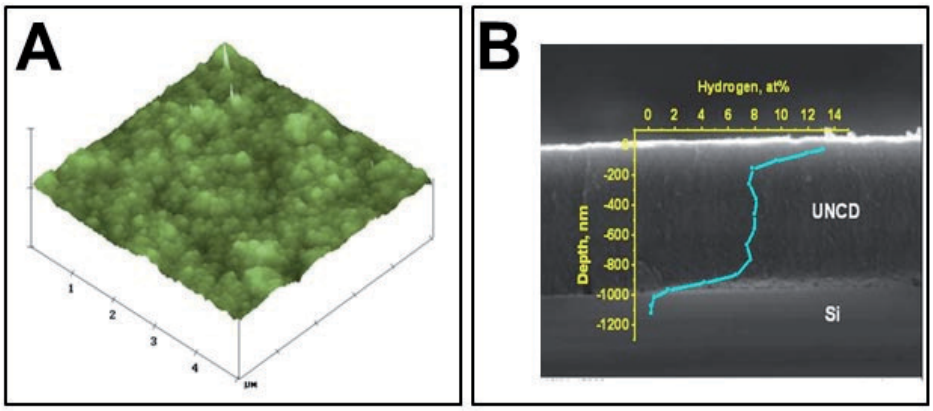

Fig. 12. AFM image of the surface of UNCD film revealing the typical topography of the coatings (A) and Hydrogen depth distribution determined by nuclear reaction analysis and scanning electron microscopy cross section micrograph of UNCD film (B) (Kulisch et al., 2008; Popov et al., 2003; Popov et al., 2011).

The surface composition of UNCD films after different modifications, described above, has been also investigated by XPS (Popov et al., 2008b) and the results are summarized in Fig. 13A. As already mentioned, the surface of *AG* is very clean, the same holds for *AR*. The sample *NP* exhibits an increase of the surface nitrogen concentration from 1.0 to 7.4 at% after ammonia treatment (Koch et al., 2011). It was found that the oxygen content of the O_2 plasma treated surface is about 12 at% (*OP*) and about 8.5 at% for the UV/O_3 treated sample (*UV*), in contrast to 2.0 at% for *AG*, indicating an oxidation of the surface by both processes. The plasma treatment with CHF_3 leads to a surface fluorine concentration of almost the same value (ca. 12 at%) showing that a fluorination process has taken place on the surface. Having in mind that the hydrogen surface concentration of the as-grown samples is about 14 at% (Kulisch et al., 2008), the XPS results indicate a change of the surface termination by the plasma processes. Closer analyses of the XPS peaks revealed that in the case of the oxygen plasma treatment the terminating hydrogen atoms are replaced by O and OH groups rather than by carboxylic acid groups (Popov et al., 2008b). CHF_3 plasma treatment has led to a substitution of the C-H bonds by C-F rather than to the deposition of a $C_xH_yF_z$ polymer (Popov et al., 2008b).
The wettability of the differently treated UNCD surfaces against purified water were examined by contact angles measurements. The results presented in Figs. 13B and 14 show

that all applied treatments have resulted in modification of the original surface. Three types of surfaces can be differentiated: hydrophobic (*AG*, *HP*, *FP*), hydrophilic (*OP*, *NP*, *UV*) and surface *AR* in between the two groups. The contact angles q_C of the three highly hydrophobic samples are in the order *AG* < *HP* < *FP* varying between 85 and 100°, while all hydrophilic samples possess q_C < 10°. The contact angle of *AR* is 67° showing also a hydrophobic character.

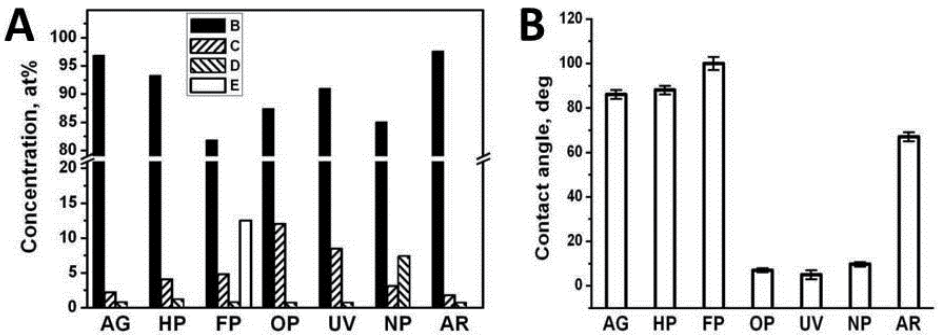

Fig. 13. Surface composition of ultananocrystalline diamond/amorphous carbon composite films after different treatments (B – carbon; C- oxygen; D- nitrogen; E- fluorine) (A) and contact angles of same surfaces after different treatments (B) (Popov et al., 2011).

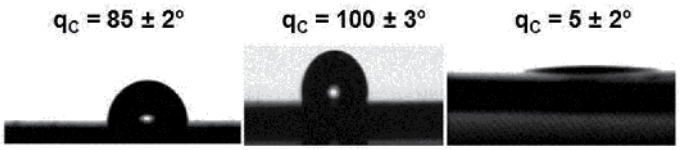

Fig. 14. Contact angles and water droplet profiles on ultananocrystalline diamond/amorphous carbon composite films: as-grown (left), after F-containing plasma modification (middle) and after UV/O_3 treatment (right) (Popov et al., 2011).

The interaction of a surface with cells is usually dominated (at least in the initial stage) by adhesion of proteins onto the biomaterial surface, which occurs rather rapidly. For this reason it is important to study the interactions of proteins with UNCD surfaces and to tailor them, if necessary, by suitable surface modifications. Up to now, three series of experiments have been carried out to investigate the UNCD/protein interactions.

In the first series, scanning force spectroscopy measurements have been performed with as-grown UNCD films and glass (as a reference) onto which proteins have been deposited. A silicon cantilever functionalized with bovine serum albumin (BSA) has been used for this purpose. 120 single measurements at different positions have been performed for each of the two samples. On the UNCD sample none of 120 measurements indicated any interaction; all force curves had the structureless shape. In contrast, for the glass sample in 38% of all force/distance measurements an interaction between the BSA-functionalized cantilever and the surface was observed, which gave rise to force curves. Thus it can be concluded that the UNCD surfaces are not prone to unspecific interactions with proteins (Popov et al., 2007).

On the other hand, even on such inactive surfaces there will be adhesion of highly fouling proteins such as BSA. In order to investigate whether the BSA adhesion on UNCD films is influenced by the surface termination, several of the above discussed surfaces have been

subjected to BSA exposure in the second series of experiments. Immediately after this exposure the surfaces were investigated by time of flight secondary ion mass spectroscopy (ToF-SIMS) and XPS (Kulisch et al., 2007). According to the ToF-SIMS analysis, all surfaces were covered by a BSA layer approaching a monolayer but it turned out to be impossible to quantify the adhesion and to evaluate differences between the various surfaces. More insight into this question was brought by XPS measurements. The surface composition of all samples investigated was far from that of a thick spin-coated BSA layer on a silicon substrate, taken as a reference, indicating that the BSA coverage on the UNCD surfaces is limited. As the composition of the starting surfaces is different as discussed above, it seems to be more useful to look at the compositional changes inferred by the BSA exposure which are shown in Fig. 15A. From this figure it is evident that the changes of the surface composition of the as-grown *AG* surface are only marginal. They are more pronounced for the H_2 and O_2 plasma treated samples (*HP* and *OP*). The largest changes, and thus the highest adhesion of BSA, were observed for the chemically aqua regia treated surface *AR*. A possible reason is the partial loss of the hydrogen termination caused by the treatment as discussed above. Summarizing, it can be stated that the unspecific adhesion of biomolecules such as the highly fouling bovine serum albumin on UNCD surfaces can be influenced by the surface termination.

In the third series of experiments, the protein adsorption on UNCD surfaces with different terminations was studied by inverted enzyme-linked immunosorbent assay (ELISA) with albumin and fibrinogen (Popov et al., 2009). The ratio of albumin to fibrinogen adsorption was calculated from the individual levels of both proteins adsorbed on the surfaces (Fig. 15B). The oxygen terminated layers exhibit a higher albumin to fibrinogen ratio as compared with the fluorine and hydrogen terminated films. It has been pointed out that the variation of the albumin and fibrinogen adsorption ratio is strongly related to the associated surface energies since fibrinogen, being itself hydrophobic, preferentially adsorbs on hydrophobic surfaces, but albumin (with a hydrophilic nature) on hydrophilic surfaces during competitive binding. The O-terminated UNCD layers have a hydrophilic surface (33° contact angle against water), leading to a higher albumin/fibrinogen ratio. The F-terminated films show the lowest protein ratio, which is related to the hydrophobic nature of these surfaces (contact angle of water 91°). Therefore the albumin adsorption is much greater on the oxygen terminated surfaces and vice versa – the fibrinogen is preferentially adsorbed on the hydrophobic fluorine and hydrogen terminated surfaces.

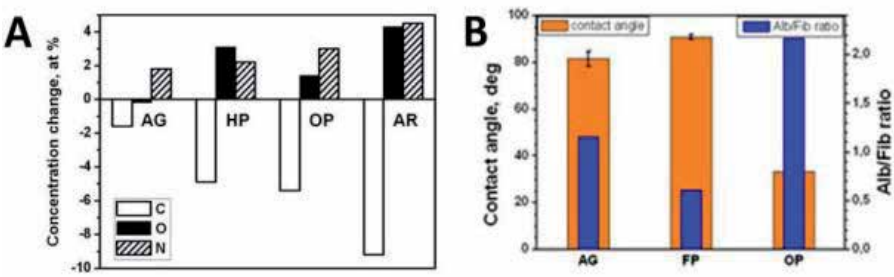

Fig. 15. Relative changes of the surface composition caused by the exposure of the samples to bovine serum albumin (A) and Contact angles and BSA/fibrinogen adsorption ratios on differently terminated UNCD surfaces (B) (Popov et al., 2011).

The biocompatibility of UNCD films was studied by direct contact tests with osteoblast-like cells, fibroblasts and endothelia cells (Popov et al., 2006a; Popov et al., 2007; Popov et al.,

2008a). All cells showed good adhesion and spreading on the UNCD surfaces following the incubation. After several days of cultivation they formed confluent monolayers; comparisons with cells from control samples showed that the UNCD films are not cytotoxic and do not affect the cell viability and proliferation.The coatings are also bioinert as revealed by simulated body fluid (SBF) tests. The exposure to SFB with a composition close to that of blood plasma for 10 days did not result in the formation of hydroxyapatite as shown by analyses of the SBF composition and of the film surface (Popov et al., 2006a).

6. A look at the future

Extraordinary progress has been made in the last decade towards the design of implants and scaffolds with a suitable multi-scale hierarchical structure. The limitations of the design of current bone implants arise mainly from the lack of firm quantitative mechanical data of bones in different stages of osteoporosis. Whilst it is known that osteoporotic bone is generally not cancellous in nature and has thin walls, the essential design paradigm of implants does not reflect this application.

A simple system for assisted bone repair proposed is the *in bone injection* of "intelligent" polymers combined with progenitor cells. It utilizes autologous stem cells transplantation in combination with supportive bioresorbable matrices and bioactive molecules for enhancing growth and repair. Ideally, endothelial progenitor cells obtained from peripheral blood of the same patient may be cultured *in vitro* in the presence of different stimuli and/or nanoparticles to undergo osteoblasts differentiation, prior to autologous transplantation. This injectable therapy could also be used for: (1) modifying the bone interior morphology, porosity and interconnectivity, which are extremely important for cell adhesion, proliferation and differentiation; (2) prophylactic treatment for high risk patients to prevent fractures, especially the hip and vertebrae; (3) treatment to stabilize loose prostheses for patients who would soon require revision surgery; (4) providing exceptional repair of the osteoporotic bone by releasing pharmaceuticals to the specific sites with the purpose of accelerating healing, promoting angiogenesis, reducing the risk of infection, etc.

The pursuit of effective treatments for osteoporotic disease is an extremely challenging scientific frontier requiring the integration of multiple engineering, biological, chemical, surgical, and pathophysiology related disciplines. It is also necessary to have a better understanding of molecular and cellular mechanisms specific to osteoporosis. Studying genomics, proteomics and diseases biology in parallel is likely to yield transformative insights in this regard. Our results together with the continuously incoming new data could have direct implications in the use of biomaterials in tissue engineering and in combination with the rapid manufacturing techniques will offer great opportunities to generate different scaffolds for bone engineering in near future.

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8. Conflict of interest

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Simulating Bone Atrophy and Its Effects on the Structure and Stability of the Trabecular Bone

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1. Introduction

According to the world health organization (WHO) osteoporosis is considered to belong to the ten most important diseases worldwide. It is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture (NIH, 2000). Osteoporosis is a metabolic bone disorder in which bones become brittle and prone to fracture. The underlying reason for the occurrence of osteoporosis is that the two processes being responsible for bone remodelling turn out of balance. Bone tissue is continuously resorbed by special bone cells, the so-called osteoclasts. On the other hand, the formation of bone also takes place by the action of other bone cells, namely the osteoblasts. These cells form new bone. The bone formation is, however, not uniform. It is rather controlled by an external mechanical stimulus such that more bone material is produced at those sites where the local stress is larger. This leads to an adaptation of the inner bone structure (trabecular bone) to externally acting forces on the bone (Mullender & Huiskes, 1995). Thereby, a minimal-weight structure, that is adapted to its applied stresses, is formed as it was already correctly conjectured by Julius Wolff as early as in 1892 (Wolff, 1892). In a healthy bone there exists an equilibrium between bone formation and bone resorption, whereas in an osteoporotic bone more bone resorption than formation takes place, which leads to a rarefied network of the trabecular bone. Besides this disease induced effect the rarefication of the inner bone structure can also have other causes like living under zero gravity conditions, immobility or age-related atrophy as the most common example.
Advances in modern imaging modalities like high resolution magnetic resonance (HRMR) or micro computed tomography (μCT) imaging have led to great improvements of the image quality especially in terms of spatial resolution which now allows for a proper three-dimensional visualisation of the trabecular network. Having these highly resolved data at

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hand it now becomes possible to perform a differential analysis of both the structural and the mechanical properties of the trabecular bone.
We determine the *local* structure of the trabecular network by calculating isotropic (α) and anisotropic scaling indices (α_x,α_y,α_z) (Räth et al., 2008). These measures have been proven to be able to discriminate rod- from sheet-like structures and to quantify the alignment of structures with respect to a preferential direction as e.g. given by the direction of the external force.
Another class of texture measures is given by Minkowski Functionals (Michielsen & De Raedt 2001), which - as the scaling indices – also incorporate correlation functions of higher orders and supply global morphological information about structures under study.
The calculation of the local mechanical properties, i.e. the load distribution inside bodies with high porosity and complex architecture is enabled by the use of the finite element method (FEM), which has become a standard tool in bone research.
Bone modelling and remodelling processes can be simulated by describing the action of the osteoblasts and osteoclasts by means of rate equations. The equations are nonlinear partial differential equations, which can thus only be solved numerically (Huiskes et al, 2000). As it is mostly the case in nonlinear systems the solutions depend very sensitively on the choice of parameters of the model, with which e.g. the onset of bone formation is controlled.
In this chapter we propose methods to simulate the effects of bone modelling on the structure and stability of the trabecular bone. We gradually deteriorate the trabecular structure by using some concept of cellular automata (Wolfram 1983, Wolfram 1984) to solve the rate equations numerically. Specifically, both the three space coordinates as well as the time is discretised. The bone resorption, which is described as a continuous process by the partial differential equations, is thus transformed to a consecutive removal of bone surface voxel, which is discrete in space and time. Having simulated bone modelling we then assess the effects of changes in the bone structure on both the structural and mechanical properties of the specimen.
We do not consider the details of the physiological mechanisms of the bone adaptation to mechanical loading and do not describe metabolic activity of the biological cells. Rather, we assume independent action of osteoclasts (bone resorbing cells) and osteoblats (bone forming cells), which is typical for bone modelling process leading to global morphological and topological changes. Among the large variety of bone modelling scenarios we concentrate on bone atrophy due to erosion of the bone surface by osteoblasts resorption activity and decrease of osteoblast formation activity, which is typical for the process of normal aging (often called primary osteoporosis).
This type of bone modelling can be simulated by random resorption of bone voxels at the interface between trabecular bone and bone marrow. We develop three numerical models for simulation of bone loss, which correspond to the different assumptions about age-related atrophy in male and female bones: thinning of the trabecular bone structure with and without preserving of topological connectivity and with preferential loss of aligned rod-like trabecular elements, which were previously identified by a scaling index analysis.

2. Material and methods

2.1 The data set

For our study we chose 17 specimens of young (50 < age < 70) patients with high maximum compressive strength (MCS > 70 Newton (N)) out of a data set (Räth et al., 2008) of 151 cylindrical specimens with a diameter of 8 mm and a length of 14 mm, which were

harvested from 73 thoracic and 78 lumbar human vertebrae. The resulting µCT grey-value images with isotropic spatial resolution of 26 µm were segmented using a low-pass filter by convolving the image with a Gaussian kernel with standard deviation 0.8 and support of 1 to remove noise and a fixed global threshold equal to 22% of the maximal grey value to extract the mineralised bone phase (Hildebrand et al 1999). After µCT scanning, the bone samples were cut to the length of 12 mm and tested by applying uniaxial mechanical compressive load using a servo-hydraulic machine (MTS 858 mini Bionix II, MTS Eden Prairie, USA) with a load cell of 1.5 kN. Maximum compressive strength (MCS) was determined in biomechanical experiment as the first local maximum of the force-displacement curve and used in correlation analysis as a golden standard for characterisation of bone strength (Eckstein et al., 2007). Main structural characteristics of the bone specimens are given in Table 1. The binarized 3D µCT images were used as a starting structure for the numerical simulations of the bone resorption process. Table 1 summarizes some main characteristics of the data set.

	age	**BV/TV**	**Tb.N.**	**Tb.Th.**	**Tb.Sp.**	**MCS [N]**
Mean value	62.2	0.13	1.10	0.15	0.87	105
Standard deviation	4.7	0.03	0.15	0.02	0.12	30

Table 1. Some characteristics of the data set: Mean and standard deviation of the age, the histomorphometric parameters bone volume *BV/TV*, trabecular number *Tb.N.*, trabecular thickness *Tb.Th.*, trabecular spacing *Tb.Sp.*, and the mechanical parameter maximum compressive strength MCS.

2.2 Structure measures

2.2.1 Minkowski functionals

Minkowski Functionals (MF) (Michielsen & de Raedt 2001) provide a global morphological and topological description of structural properties of multidimensional data. In this method binarized images are considered as a union of 3D convex bodies (voxels)

$$I = \bigcup_{i=1}^{N_{bone}} \vec{p}_i(x_i, y_i, z_i)$$

According to integral geometry an *n*-dimensional body can be completely characterized by *n+1* functionals, which evaluate both size and shape of the object. In a three-dimensional space the four functionals are represented by the volume (MF_1), surface area (MF_2), integral mean curvature (MF_3) and integral Gaussian curvature (MF_4). Minkowski Functionals are derived from the theory of convex sets and expressed as volume integral for MF_1 and surface integrals over boundary S of the excursion set I with the principal radii of curvature R_1 and R_2 for other functionals.

$$MF_1 = \int_I dV \qquad MF_3 = 1/2 \int_{\partial I} (\frac{1}{R_1} + \frac{1}{R_2}) dS$$

$$MF_2 = \int_{\partial I} dS \qquad MF_4 = \int_{\partial I} \frac{1}{R_1 R_2} dS$$

The first two functionals MF_1 and MF_2 describe morphology of the structure and coincide with morphometrical parameters bone volume and surface fractions ($MF_1 = BV/TV$ and $MF_2 = BS/TV$). The fourth integral, also known as Euler characteristic χ, characterises the topological connectivity of structures and can be expressed in terms of Betti numbers β_0 (number of connected components), β_1 (number of tunnels), β_2 (number of cavities):

$$\chi = \beta_0 - \beta_1 + \beta_2 .$$

In the case of binary images we have exactly four global characteristics, which can be used as texture measures for assessment of bone strength (Monetti et al., 2011).

2.2.2 Isotropic and anisotropic scaling indices

We calculate isotropic and anisotropic scaling indices (SIM) (Räth & Morfill 1997; Monetti et al. 2004; Müller et al. 2006; Räth et al. 2008) as measures to characterize the complex trabecular network and its degree of alignment relative to a preferential direction.
Generally, scaling indices represent one way to estimate the local scaling properties of a n-dimensional point set. Considering binarised three-dimensional µCT-images, a suitable representation of the image information as a set of three-dimensional points is given by

$$\vec{p}_i = (x_i, y_i, z_i) ,$$

i = 1, . . . , N_{bone}, where N_{bone} denotes the number of (white) bone voxels and x_i, y_i, z_i the voxel position. The three-dimensional image can now be regarded as a set of N points

$$P = \{\vec{p}_i\}, i = 1,...,N_{bone} .$$

For each (bone) voxel the logarithmic gradients

$$\alpha_i = \frac{\partial \log \rho(\vec{p}_i, r)}{\partial \log r} ,$$

which are called scaling indices, of the cumulative weighted point distribution

$$\rho(\vec{p}_i, r) = \sum_{j=1}^{N_{bone}} e^{-\left(\frac{d_{ij}}{r}\right)_n}$$

are calculated with

$$d_{ij} = \left\| \vec{p}_i - \vec{p}_j \right\|_2 = \left((x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2 \right)^{1/2}$$

for the isotropic case.
The calculation of the scaling indices depends on the parameters n and r. The exponent n controls the shape of the weighting function. With increasing n the weighting function becomes more and more step-like. In all our studies we found that n has only little influence on the results. For the following calculations we fixed n to n=2, which emphasises the connection to Gaussian smoothing or kernel functions. The scale parameter r controls to which scales of the image structure the scaling indices are sensitive. From previous studies

we found that r = 8 (in voxel units) is a well-suited choice. By means of the scaling indices α one can then discriminate between voxels belonging to rod-like (α ≅ 1) and sheet-like (α ≅ 2) structural elements of the trabecular network, which is one of most important discriminating feature to discern healthy from osteoporotic bone structure.
It is straightforward to implement anisotropies in the calculation of the scaling indices by introducing an ellipsoidal distance measure with the eigenvalues λ_x, λ_y and λ_z:

$$d_{ij} = \left\| \vec{p}_i - \vec{p}_j \right\|_{\lambda_x,\lambda_y,\lambda_z} = \left(\lambda_x^2 (x_i - x_j)^2 + \lambda_y^2 (y_i - y_j)^2 + \lambda_z^2 (z_i - z_j)^2 \right)^{1/2}$$

In this study we calculate anisotropic scaling indices α_z with respect to the direction of the external force acting in the bone, for which we chose the ratio of 5:1 for the eigenvalues, i.e. $\lambda_z = 5\lambda_x = 5\lambda_y$, for setting the degree of anisotropy along the z-direction. This setting was found to be well suited especially for the detection of aligned cylindrical structures (i.e. the trabeculae) with typical mean breadth and length.

2.3 Finite element models

For assessing biomechanical strength of trabecular network we apply Finite Element Method (FEM) with linear elastic assumptions (Rietbergen et al., 1995; Rietbergen et al., 1999; Sidorenko et al. 2009). For the bone mineral material we assume isotropic properties with Young's modulus $Y = 10\ GPa$ and Poisson's ratio $\nu = 0.3$ and describe relationship between stress σ and strain ε components by generalized Hooke's law (constitutive equations), which states that stress is proportional to the strain up to the elastic limit

$$\sigma_{ij} = 2\mu\varepsilon_{ij} + \lambda\delta_{ij}\sum_{k=1}^{3}\varepsilon_{kk} .$$

Material properties are included in the model through Lame parameters λ and μ

$$\{Y,\nu\} \Rightarrow \left\{ \lambda = \frac{Y\nu}{(1+\nu)(1-2\nu)}, \quad 2\mu = \frac{Y}{1+\nu} \right\}$$

We use Dirichlet boundary conditions with prescribed on the top surface constant strain $\varepsilon_0 = 1\%$ to simulate uniaxial loading in the natural direction (in this work denoted as z axis) applied in biomechanical experiment. We generate a finite element mesh by direct converting image voxels that belong to the bone tissue into equally sized and oriented eight node brick elements. An exact number of nodes depend on the structure characteristics and varies for our data set from $0.7 \cdot 10^6$ for weak bones, dominated by rod-like trabecular elements, up to $2 \cdot 10^6$ for strong bones with a lot of plate–like formations. From discrete nodal displacements obtained from FEM strain and stress components can be recovered at any point of the structure. For correlation analysis with respect to the MCS we use apparent total reaction force F_r at the top face A^t which is recalculated from the normal stress component in direction of applied mechanical load σ^t_{zz}:

$$F_r = \int \sigma^t_{zz} dA^t .$$

2.4 Bone resorption models

We develop three numerical models for bone resorption, which correspond to the different assumptions about bone atrophy in male and female bones. Several investigations (Aaron et al., 1987; Khosla et al., 2006) demonstrated sex difference in trabecular bone aging. Although the decrease with age in trabecular bone volume is common for both sexes, male cancellous bone exposes uniform thinning, whereas female trabecular network suffers from loss of connectivity and entire rod-like structural elements (Barger-Lux et al., 2002).

In all three models we simulate the random resorption of bone material by removal surface voxels according to the given value of relative bone loss volume $\Delta BV/TV$, but with different topological features. In order to study the role of topological connectivity for bone strength in the first resorption model (Model I), a bone voxel is removed only if does not change topology of the system, i.e. no new cavities or tunnels are created. In terms of global topological characteristics it means that the fourth Minkowski Functional, which coincides with Euler characteristic χ is conserved,

$$MF_4 = \chi = \text{const.}$$

We compare this model with two other models, which both do not preserve the connectivity ($\chi \neq const$). In Model II there isn't any limitations or conditions on the removal of bone surface voxels. In Model III, however, we simulate the preferential destruction of rod-like trabecular elements by only removing surface voxels with local topological anisotropic scaling index $\alpha_z < 2$. Typical changes in trabecular bone structure are demonstrated in Fig. 1. Three regions in circles show the differences in structure due to different resorption models: preserving of connectivity and destroying of rod-like elements at the same place in the trabecular network.

3. Results

As a main characteristic of the statistical analysis we use Pearson's correlation coefficient r_{MCS} (Tables 2 - 5) with respect to the Maximum Compressive Strength (MCS) as it was measured in biomechanical uniaxial compressive experiments. In the Figs. 2 - 4 we show the changes of the different texture measures (black curves and left axis) calculated by MF, SIM and FEM as a function of bone loss. The red curves and right axis in the Figs. 2 - 4 and the values in the corresponding Tables 2 - 4 denote Pearson's correlation coefficient r_{MCS} as function of bone loss for the three resorption models.

The diagrams for the first Minkowski Functional $MF_1 = BV/TV$ (Fig. 2, first row) confirm the linear decrease in bone mineral volume due to resorption and thus represent a validation of our implementation of the bone loss models.

Plots for $MF_4 = \chi$ (Fig. 2, last row) proof that in first resorption model (left column) the connectivity is preserved ($\chi = const$) and in the two other models the porosity of the structure increases ($\Delta\chi < 0$) during the resorption process. In both models the increase of porosity occurs due to increase of number of tunnels (β_1). In the third model with preferential resorption of rod-like trabecular elements (with $\chi \neq const$ and $\alpha_z < 2$) the growth of number of tunnels (β_1) is compensated by an increase of number of separate parts (β_0) and slows down negative increase of χ. For both resorption models without conservation of connectivity for structure with large relative bone loss ($\Delta BV/TV > 30\%$) the correlation coefficient of $MF_4 = \chi$ with MCS becomes considerably higher than that for original structure (Tables 2).

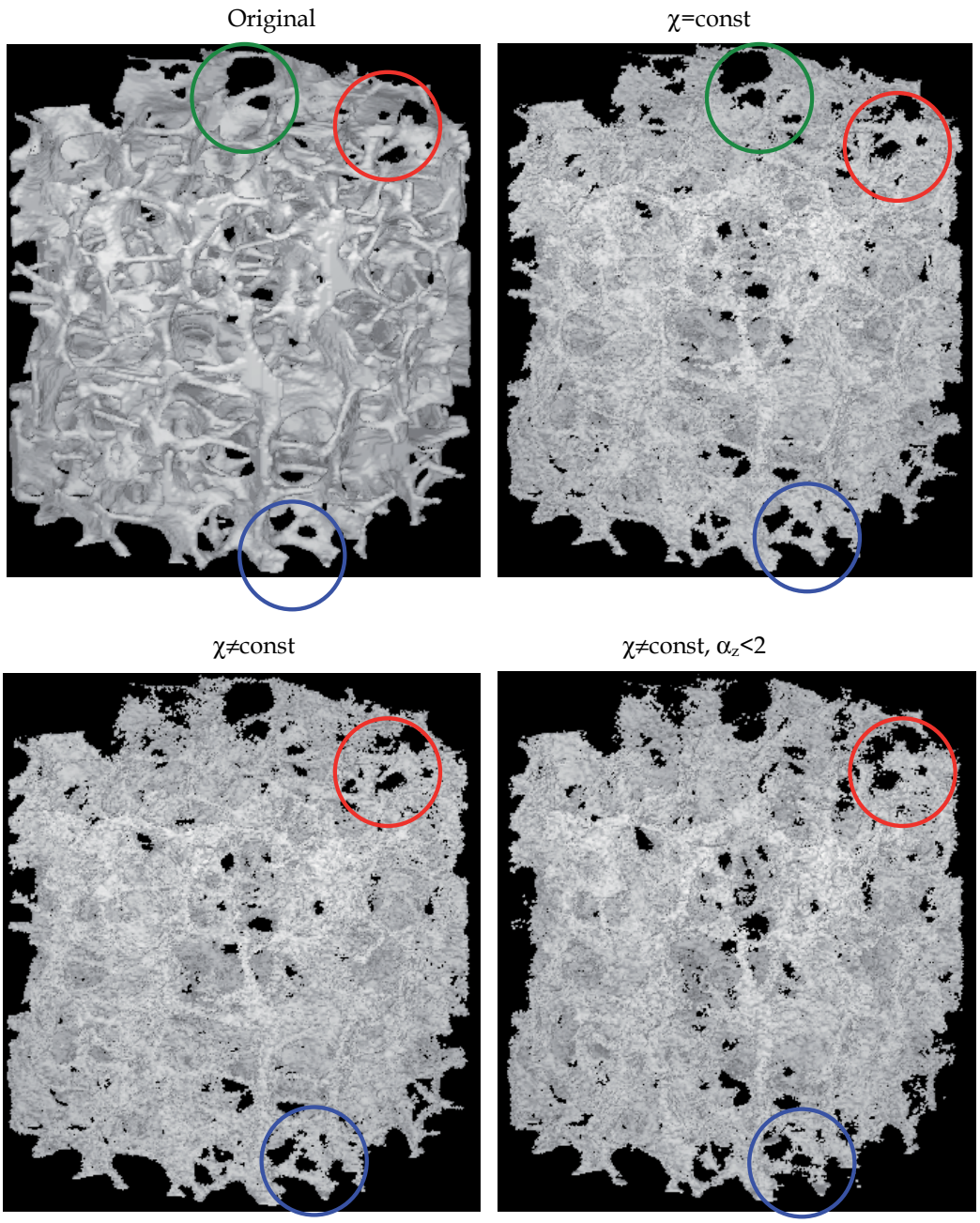

Fig. 1. Typical change of the trabecular bone structure under numerically simulated bone resorption (upper left: original bone, upper right: connectivity preserving model, lower left: model without preserving connectivity, lower right: model with preferential resorption of rod-like trabecular elements).

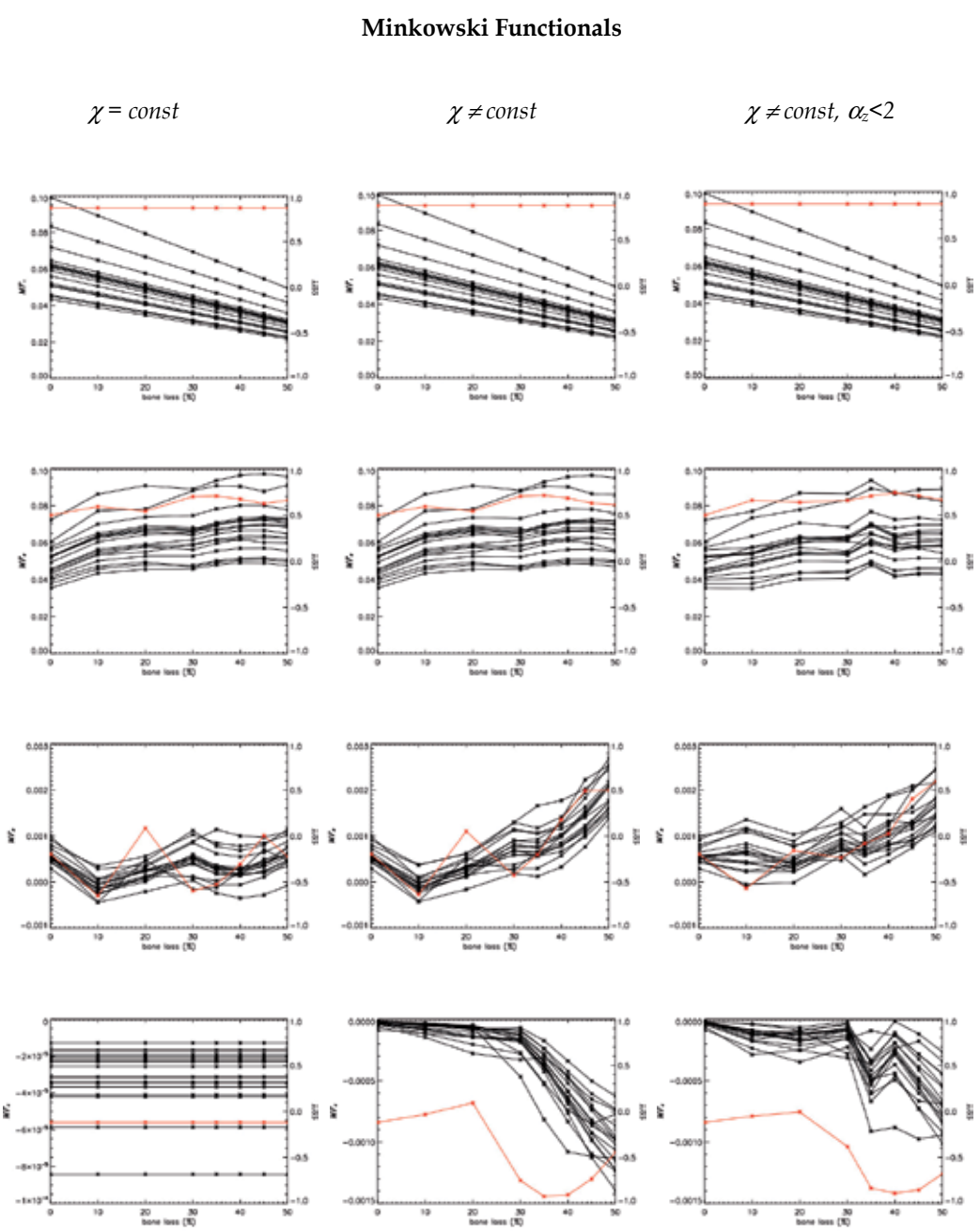

Fig. 2. Minkowski Functionals for 17 specimens (black curves and left axis) and correlation coefficient with MCS (red curve and right axis) for three models of bone loss (from left to right).

Model I

	MF_1	**MF_2**	**MF_3**	**MF_4**
0%	0.87	0.50	-0.20	-0.12
10%	0.87	0.59	-0.65	-0.12
20%	0.87	0.54	0.09	-0.12
30%	0.87	**0.70**	-0.60	-0.12
35%	0.87	**0.71**	-0.53	-0.12
40%	0.87	0.67	-0.31	-0.12
45%	0.87	0.62	0.0	-0.12
50%	0.87	0.66	-0.22	-0.12

Model II

	MF_1	**MF_2**	**MF_3**	**MF_4**
0%	0.87	0.50	-0.20	-0.12
10%	0.87	0.59	-0.64	0.01
20%	0.87	0.54	0.05	0.09
30%	0.87	**0.70**	-0.43	**-0.75**
35%	0.87	**0.71**	-0.20	**-0.92**
40%	0.87	0.68	0.18	**-0.91**
45%	0.87	0.63	**0.49**	**-0.73**
50%	0.87	0.61	**0.50**	**-0.45**

Model III

	MF_1	**MF_2**	**MF_3**	**MF_4**
0%	0.87	0.50	-0.20	-0.12
10%	0.87	0.66	-0.57	-0.05
20%	0.87	0.64	-0.16	-0.01
30%	0.87	0.66	-0.25	**-0.38**
35%	0.87	**0.70**	-0.08	**-0.83**
40%	0.87	**0.74**	0.03	**-0.89**
45%	0.87	**0.71**	**0.40**	**-0.85**
50%	0.87	0.66	**0.59**	**-0.68**

Table 2. Pearson's correlation coefficient of the four MF with respect to MCS for the three models of bone loss and eight rarefiication steps ranging from 0% to 50% removal of the initial bone volume.

For isotropic SIM (Fig. 3a) there is almost no difference in the $P(\alpha)$ spectrum for the different resorption models observed, which leads to only a small decrease in the correlation coefficient (last row in Fig. 3a and Table 3a). For anisotropic SIM (Fig. 3b) there is an obvious difference in $P(\alpha_z)$ already at $\Delta BV/TV = 10\%$ and a more considerable decrease of r_{MCS} (last row on Fig.3b and Table 3b) in the case of preferential resorption of rod-like trabecular elements (third model with $\chi \neq const$ and $\alpha_z < 2$).

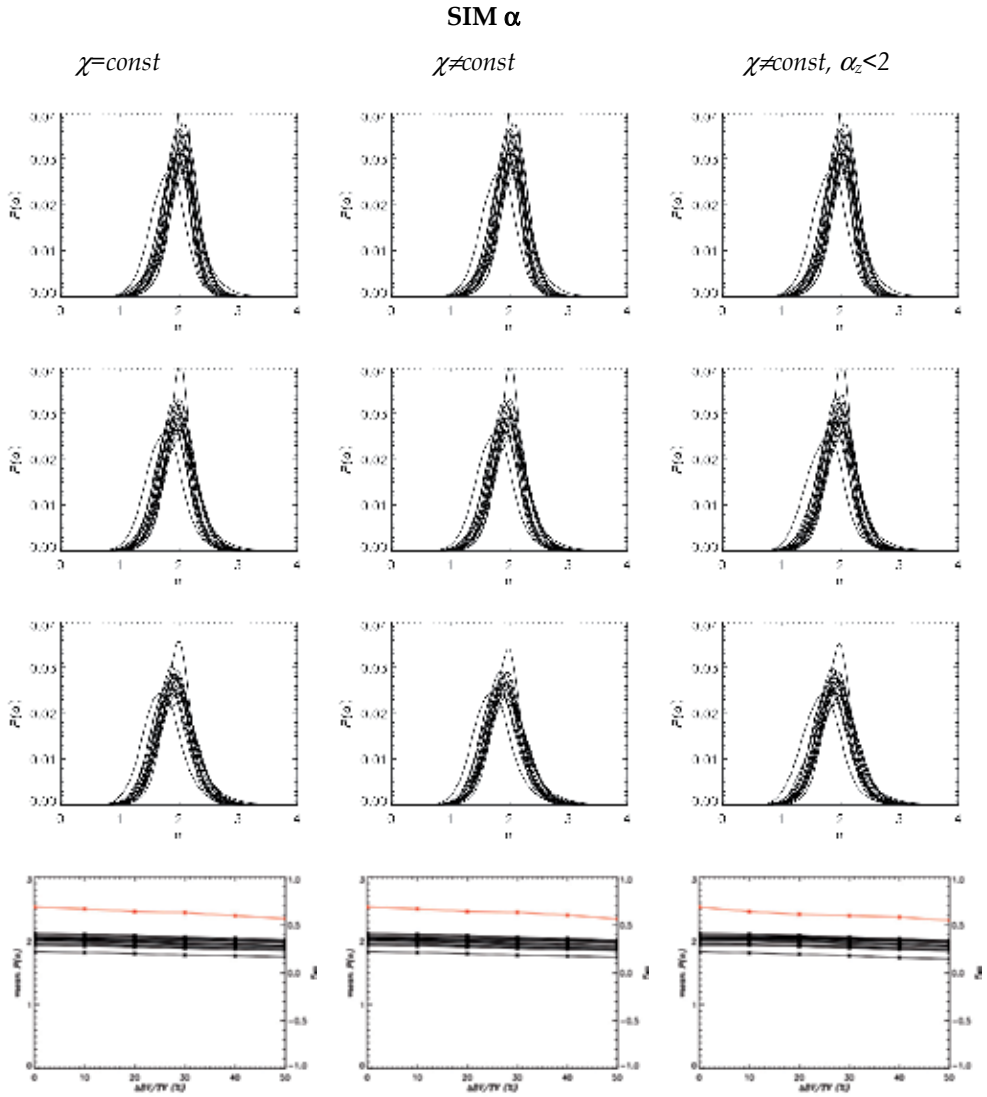

Fig. 3a. Probability distribution function of isotropic scaling index $P(\alpha)$ for 17 bone specimens with original structure (first row) and with different value of bone resorption (30%: second row, 50% third row). Last row: mean value of α spectrum (black curves and left axis) and correlation coefficient of mean value with MCS (red curve and right axis) for three models of bone loss (from left to right).

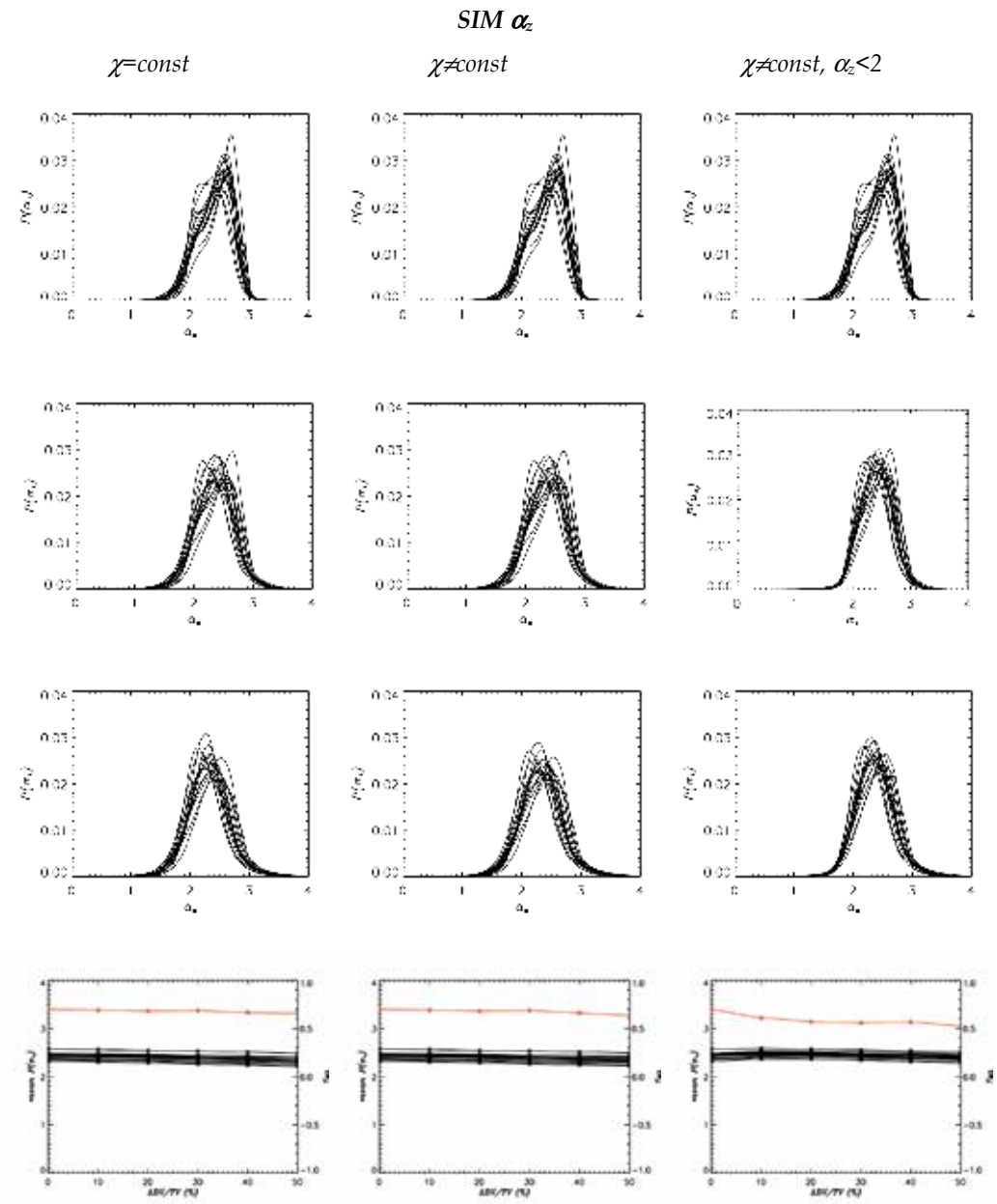

Fig. 3b. Probability distribution function of anisotropic scaling index $P(\alpha_z)$ for 17 bone specimens with original structure (first row) and with different value of bone resorption (30%: second row, 50% third row). Last row: mean value of α_z spectrum (black curves and left axis) and correlation coefficient of mean value with MCS (red curve and right axis) for three models of bone loss (from left to right).

	χ=const	χ≠const	χ≠const, α_z<2
0%		0.69	0.69
10%	0.67	0.67	0.64
20%	0.64	0.64	0.61
30%	0.63	0.63	0.60
40%	0.60	0.60	0.58
50%	0.57	0.56	0.55

a)

	χ=const	χ≠const	χ≠const, α_z<2
0%	0.70	0.70	0.70
10%	0.69	0.69	0.61
20%	0.68	0.68	0.57
30%	0.68	0.68	0.56
40%	0.66	0.66	0.56
50%	0.66	0.64	0.52

b)

Table 3. Correlation coefficient of mean value of α spectrum *(a)* and α_z spectrum *(b)* with MCS for three models of bone loss.

The mechanical strength of the resorbed trabecular structure as determined with FEM (Fig. 4) depends almost linearly on the relative bone loss $\Delta BV/TV$ and shows small decrease in the correlation with MCS (Table 4). At high bone loss ($\Delta BV/TV = 50\%$) the mechanical strength of all bone specimens was found to be larger in the case with conservation of connectivity (solid line in Fig. 5). In Table 5 we summarise effect of large bone loss ($\Delta BV/TV = 40\%$) on different numerical texture measures. FEM and SIM demonstrate small drop in correlation with MCS of initial structure. These methods can be proposed for prediction of osteoporosis: relative strength and local topology do not change considerably under the process of random surface resorpton. In a contrast, after large bone resorption global MF_2 and especially MF_4 improve their correlation with MCS of the original structure. This effect can be used for the diagnostic of the current state of the bone structure.

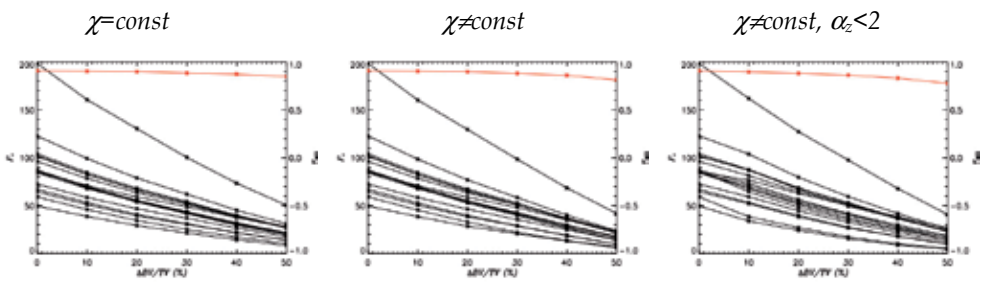

Fig. 4. Value of the apparent top reaction force F_r for 17 bone specimens calculated by FEM (black curves and left axis) and correlation coefficient of F_r with MCS (red curve and right axix) for three different resorption models (from left to right).

	χ=const	χ≠const	χ≠const, α_z<2
0%	0.91	0.91	0.91
10%	0.91	0.91	0.89
20%	0.90	0.90	0.88
30%	0.89	0.88	0.86
40%	0.87	0.86	0.83
50%	0.85	0.82	0.77

Table 4. Correlation coefficient of apparent top reaction force F_r calculated by FEM with MCS for three models of bone loss.

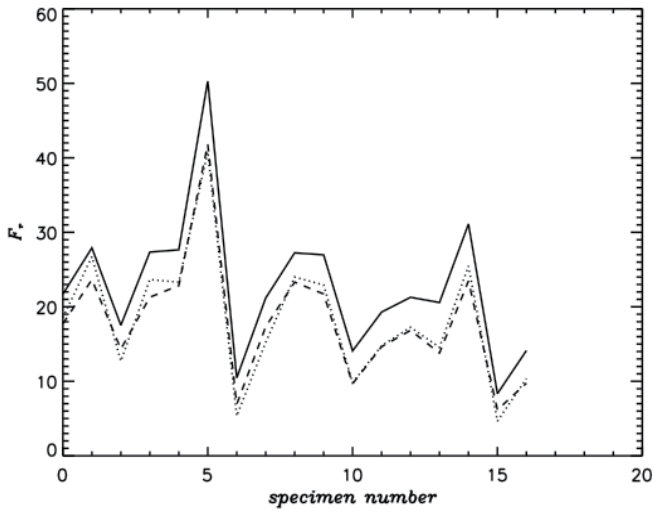

Fig. 5. Apparent total reaction force F_r for 17 bone specimens and for the three resorption models with $\Delta BV/TV=50\%$ (solid line: $\chi=const$, dashed line: $\chi\neq const$, dotted line: $\chi\neq const$, $\alpha_z<2$.

	original	$\chi=const$	$\chi\neq const$	$\chi\neq const$, $\alpha_z<2$
FEM	0.91	0.87	0.86	0.83
MF_1	0.87	0.87	0.87	0.87
MF_2	0.50	0.67	0.68	0.74
MF_3	-0.20	-0.31	0.18	0.03
MF_4	-0.12	-0.12	-0.91	-0.89
Mean $P(\alpha)$	0.69	0.60	0.60	0.58
Mean $P(\alpha_z)$	0.70	0.66	0.66	0.56

Table 5. Pearson's correlation coefficient with respect to the MCS for bone structure with $\Delta BV/TV=40\%$ for original structure and three resorption models

4. Summary and conclusions

We proposed a method based on the ideas of cellular automata to simulate bone atrophy and applied it to a sample of 17 bone probes visualised with high resolution μCT imaging. Although our study is so far restricted to the simulation of bone loss and did not include any processes of bone formation, we could already gain some new and very interesting insights about the important factors determining the strength of bones.

As expected we found that the initial structure determines the relative strength of the bone under random surface bone losses. Patients with stronger bones in young age have better prognosis for age-related bone atrophy. We found that the connectivity plays the most important role in determining the strength of the bone structure: among three resorption models the highest apparent reaction force was calculated for the resorption model which preserved the connectivity ($\chi = const$). FEM, isotropic SIM, the first and second MF yielded stable values of the correlation coefficient r_{MCS} under the random bone loss process for all numerical resorption models and can be recommended for prediction of bone strength in bone atrophy process.

The mean value of the anisotropic scaling indices α_z demonstrated sensibility for preferential rod-like trabecular loss as simulated by third numerical resorption model (with $\chi \neq const$ and $\alpha_z < 2$). For this model the scaling index approach shows a decrease of correlation coefficient r_{MCS} already at 10% loss of mineral bone fracture.

For the two resorption models without conservation of the connectivity ($\chi \neq const$) the bone surface resorption significantly improves the correlation of the fourth MF with MCS (from $r_{MCS} = -0.12$ for original structure up to the $r_{MCS} = -0.92$ for bone loss ratio $\Delta BV/TV = 35\%$). Such an effect suggests that the random surface resorption destroys thin and unimportant connections of the trabeculae and only the strong and thick trabecular elements are taken into account for correlation with MCS. The removal of bone voxels can thus be interpreted as a distillation of the essential skeleton of the trabecular structure, which is a much more sensitive tracer of the mechanical stability of the bone. In fact we found that fourth Minkowski Functional calculated for structure prepared by random surface resorption yields higher correlations with MCS than FEM-based measures, which are so far considered to yield the highest correlations with the mechanical properties of bone probes. Therefore the rarefication procedure as outlined in this study in combination with Minkowski Functionals may lead to a novel technique for the diagnosis of the trabecular bone quality and strength in the prediction of osteoporosis.

5. Acknowledgments

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Role of Phytoestrogen Ferutinin in Preventing/Recovering Bone Loss: Results from Experimental Ovariectomized Rat Models

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1. Introduction

Osteoporosis is a chronic bone disease, caused by an imbalance between bone resorption and bone formation (Riggs & Melton, 1986), in which the skeleton becomes fragile and leads to an increased risk of fractures. In osteoporosis the bone mineral density is rapidly reduced, the bone microarchitecture is disrupted and the amount/variety of non-collagenous bone proteins is altered (Chestnut, 1995; Paschalis et al., 1997). In menopausal women, the rapid decrease of estrogens is the predominant cause of the imbalance between bone formation and bone resorption that results, in turn, in severe bone loss (Riggs & Melton, 1986). Hormone Replacement Therapy (HRT), based on estrogen administration, is a method to recover both bone loss and incidence of skeletal fractures in postmenopausal women (Turner et al., 1994); however, as it is well known, it increases, as negative side effects, the occurrence of cardiovascular diseases and endometrial/breast/ovarian malignant cancers (Beral, 2003; Genant et al., 1998; Lacey et al., 2002; Termine & Wong, 1998). In addition to HRT, other compounds such as bisphosphonates, calcitonin, calcium products, RANK Ligand, stronzium ranelate, PTH 1-34, thiazide diuretics and ipriflavone (Bruhn, 2010; El-Desoky et al., 2009; Lacroix, 2000; Rybchyn et al., 2011; Schoofs, 2003; Wasnich, 1983; Zhang et al., 2010) are currently used as pharmacological approaches to osteoporosis, but they are often associated with negative side effects. Therefore, the need to find safer and more effective bone protective agents is still prominent. A great number of preparations from medicinal plants was shown to reduce bone loss induced by ovariectomy in rats (Occhiuto et al., 2007) and to increase bone density in postmenopausal women (Clifton-Bligh, 2001). Among natural products increasingly used as an alternative therapy, the phytoestrogenic isoflavones have been shown to increase bone density in postmenopausal women following high dietary intake (Mei et al., 2001). Also in animal studies, the administration of isoflavones or their derivatives prevented bone loss in ovariectomized rats. They are structurally similar to estradiol and their estrogenic-like activity may also depend on their affinity for some estrogen receptors (ERs). Phytoestrogens appear to bind preferentially to the ERβ and have been classified as Selective Estrogen Receptor Modulators (SERMs) (An et al., al., 2001; Brzezinski & Debi, 1999; Messina et al., 2006). ERβ may play a protective role in breast breast cancer development by reducing mammary cell growth, as well as inhibiting the

stimulatory effects of ERα(An et al., 2001; Strom et al., 2004). Considering the properties of such such natural compounds, phytoestrogens could be employed as Complementary/Alternative Medicine (CAM) instead of HRT, in order to recover menopausal symptoms (Lee et al., 2000; Morris et al., 2000; Morris et al., 2006). Such evidence that SERMs mime estrogens as osteoprotective substances (Albertazzi, 2002; Wang et al., 2006) without displaying the negative side effects on the etiogenesis of some types of cancer (Duffy et al., 2007; Eason et al., 2005; Gallo et al., 2006; Garcia-Perez et al., 2006; Lian et al., 2001; Limer & Speirs, 2004; Murray et al., 2003; Wu et al., 2002) suggests interesting perspectives in planning alternative treatment strategies.

A great number of preparations from medicinal plants, including red clover, hops and black cohosh, have been tested to investigate their influence on ovariectomy-induced bone loss. Red clover (*Trifolium pratense L.*) was shown to reduce bone loss induced by ovariectomy in rats (Occhiuto et al., 2007) and to increase bone density in postmenopausal women (Clifton-Bligh, 2001). The prenylated flavanone contained in hops (*Humulus lupulus L.*), 8-prenylnaringenin (8-PN), and genistein (found in a number of plants including lupin, fava beans, soybeans, kudzu, and psoralea) seem to protect from ovariectomy-induced bone loss in rats, while exhibiting minimal trophic effects on uterus endometrium (Garcìa-Pérez et al., 2006; Hümpel et al., 2005); in particular isoflavone genistein, by enhancing uterine endometrial glandular apoptosis *in vivo* and *in vitro*, may confer protection against uterine carcinoma (Eason et al., 2005). Moreover, a reduced bone resorption was demonstrated also after black cohosh (*Cimicifuga racemosa L.*) therapy and it was ascribed to the significant binding of its components to estrogen receptors (Wuttke et al., 2003). Despite the huge amount of data published *in vitro* and *in vivo* on another phytoestrogen, Ferutinin, extracted from *Ferula hermonis root* (Fig. 1) (Abourashed et al., 2001), whose effect was investigated on calcium-related cellular processes, few observations are reported in literature concerning ferutinin role on the skeleton, particularly on bone metabolism in both the preventing and curative treatment of osteoporosis. Ferutinin shows high affinity for both subtypes of estrogen receptors (ERs). Even if ferutinin can bind to both ERs, it acts as an agonist for ERα and as agonist/antagonist for ERβ(Ikeda et al., 2002). Thus, this compound may be useful as a selective estrogen receptor modulator (SERM) (Appendino et al., 2002).

Recently, the interest of the authors was to investigate the effects of ferutinin administration on bone metabolism in prevention and in recovery of severe estrogen deficiency-osteoporosis and to compare them with those of estradiol benzoate treatment, in order to propose an alternative solution to the hormone replacement therapy (HRT) commonly used in osteoporotic women. The animal model used, i.e. ovariectomized rat, appears to be an appropriate model for collecting information which could be applied to human postmenopausal osteoporosis, because of the many similarities of the pathophysiological mechanisms (Comelekoglu et al., 2006; Kalu, 1991; Wronski & Yen, 1991).

Further crucial problem correlated to the use of the phytoestrogen ferutinin is to evaluate its side effects, specifically on the organs which are reputed to be the target of estrogen effects, like uterus, vagina, mammary glands. It is well-known that estrogens stimulate endometrial proliferation and their administration in HRT was associated to an increased risk of cancer. Some phytoestrogens are claimed to have beneficial effects with a minor incidence of undesired side effects in comparison with estrogen therapy. Proliferative activity in estrogen-responsive cells can be either enhanced or suppressed by phytoestrogens depending on their concentration and relative potency (Whitten & Patisaul,

2001). Clinical reports about phytoestrogen effect on endometrial cancer are limited to case-controlled observational studies (Johnson et al., 2001). Hence the interest of the authors also in the problem of ferutinin side effects.

Fig. 1. *Ferula Hermonis*.

2. Methods

The authors report the following methods from some animal experiments they performed in the recent past on the topic.

2.1 Experimental animals and treatments

For animal experiments female Sprague-Dawley rats, aged 7 weeks and weighing 170-190 g at the beginning of the experiments, were used, according to the general age-models

reported in literature (Fanti et al.,1998; Kalu, 1991). They were housed two per cage and maintained in standard conditions with a 12:12 light/dark cycle, at the temperature of 22 ± 1°C and 55-60% relative humidity. Commercial rat pellets free of estrogenic substances and drinking water were available ad libitum. After a 7-day adaptation period, the animals were randomly divided in different groups according to two protocols (for prevention and recovery of bone loss, respectively). Animal care, maintenance and surgery were conducted in accordance with the Italian law (D.L. n. 116/1992) and European legislation (EEC n. 86/609). The experimental designs and procedures received the approval of the Bioethical Committee of the Italian National Institute of Health.

2.1.1 Preventing study protocol

The animals were randomly divided in four groups (Palumbo et al., 2009). Rats of group 1 were sham-operated, while rats of other groups were bilaterally ovariectomized (OVX) under ketamine hydrochloride plus xylazine hydrochloride anaesthesia and the ovaries were bilaterally removed; sham-operation was performed in the same way as ovariectomy, but only exposing the ovaries. Starting on the day after the ovariectomy, half of the female rats were submitted to the following treatments for 30 days and the remaining half for 60 days:
Group 1 (SHAM): Sham-operated controls receiving vehicle (5% Tween 80 in water)
Group 2 (C-OVX): Ovariectomized controls receiving vehicle (5% Tween 80 in water)
Group 3 (F-OVX): Ovariectomized treated with ferutinin 2 mg/kg/day
Group 4 (EB-OVX): Ovariectomized treated with estradiol benzoate 1.5 µg/rat twice a week.
Ferutinin, whose formula is showed in Figure 2, was solubilized in Tween 80 (5%) and deionized water and administered in the volume of 5 ml/kg by oral gavage (*per os*). The dosage was chosen taking into account previous studies on rat sexual behavior (Zanoli et al., 2005; Zavatti et al., 2006). Control animals (groups 1 and 2) received the same volume of vehicle solution. Estradiol benzoate, used as a reference compound, was dissolved in peanut oil and subcutaneously injected in the volume of 0.3 ml/rat.

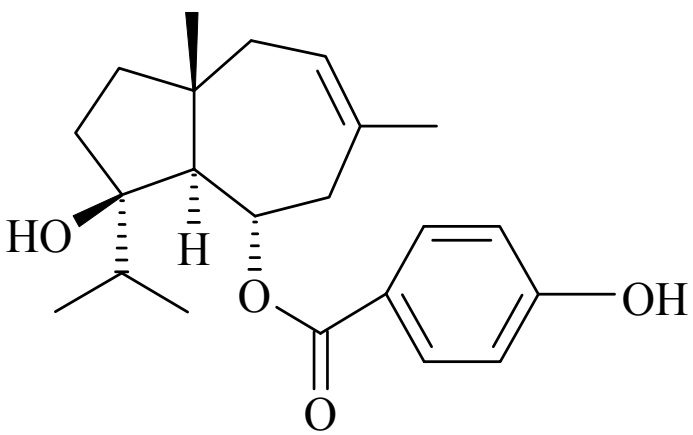


Fig. 2. Chemical structure of ferutinin.

The body weights of all animals were recorded before ovariectomy and after 30 and 60 days of treatment. Half of each rat group was sacrificed after 30 days of treatment and the remaining animals at the end of the experiment.

2.1.2 Recovering study protocol

The rats was randomized into four groups (Ferretti el al., 2010). One group of rats were sham operated, while the rats of the other three groups were ovariectomized. Ovariectomy and sham-operation were performed as above described in the protocol for preventing study. Two months after ovariectomy, namely when osteoporosis was obtained by the consequent estrogen deficiency, half of the rats of each group underwent the following treatments for 30 days and the remaining ones for 60 days:
Group 1 (SHAM): Sham-operated controls receiving vehicle (5% Tween 80 in water)
Group 2 (C-OVX): Ovariectomized controls receiving vehicle (5% Tween 80 in water)
Group 3 (F-OVX): Ovariectomized treated with ferutinin 2 mg/kg/day
Group 4 (EB-OVX): Ovariectomized treated with estradiol benzoate 1.5 μg/rat twice a week.
Ferutinin and Estradiol benzoate were used as above described.
The body weight of each animal was recorded at 4 different times: before ovariectomy (i.e., at the start of the experiment), two months after ovariectomy (namely, at the beginning of treatment), and after 30 and 60 days of treatment. At the end of the treatments, all rats were sacrificed.

2.2 Histology and histomorphometrical evaluation

Soon after the sacrifice, the 4th, 5th lumbar vertebrae and the right femurs were removed, processed and embedded in methyl methacrylate according to standard protocol for light microscopy. Serial sections of 200μm thickness were taken from both lumbar vertebrae and femurs by means of a diamond-saw microtome cutting system. In particular, the 4th lumbar vertebrae were cut according to sagittal planes, whereas the 5th lumbar vertebrae were transversely cut; concerning the femurs, the distal epiphyseal level was sagittally sectioned, whereas the shaft was transversely sectioned at the mid-diaphyseal level (Fig. 3).
Histomorphometric analysis was performed on Fast-Green or Alizarin-Red stained sections using a light microscope equipped with an image analysis system. In histomorphometric evaluations of vertebral bodies, only trabecular bone was taken into account: it was manually selected, outlining the internal surface of the cortical bone (Fig. 3A,B). In femoral sagittal sections, a constant area (3.5mm^2) of trabecular bone was defined by drawing a circular line adjacent to the cartilagineous plate (Fig. 3C). In transversal mid-diaphyseal femoral sections the cortical bone area was measured (Fig. 3D).
The following parameters were calculated:

- the ratio between the *trabecular bone area* (BV) and the *total area* (TV), i.e. the *trabecular bone volume* (BV/TV) expressed in percent values, in trabecular bone;
- the difference between the total cross section area and the medullary canal area , i.e. the *cortical bone area* (Ct-B-Ar), in cortical bone.

Only in preventing study protocol, in order to obtain a more precise evaluation of the collected data (i.e. to eliminate the effects of body weight on bone histomorphometric parameters), both the ratio BV/TV and the value Ct-B-Ar were "normalized" (i.e. corrected) with respect to body weight (dividing the calculated parameters by the body weight) on the basis that ovariectomy implies a considerable weight increase, while the chronic treatment with both ferutinin and estradiol benzoate (starting the day after ovariectomy) avoids such increment. On the contrary, in recovering study protocol the same treatment was performed 2 months after ovariectomy and after such period, the body weights of all OVX animals (C-OVX, F-OVX, EB-OVX) were all similar; for this reason, histomorphometric parameters (BV /TV and Ct-B-Ar) were not normalized with respect to body weight.

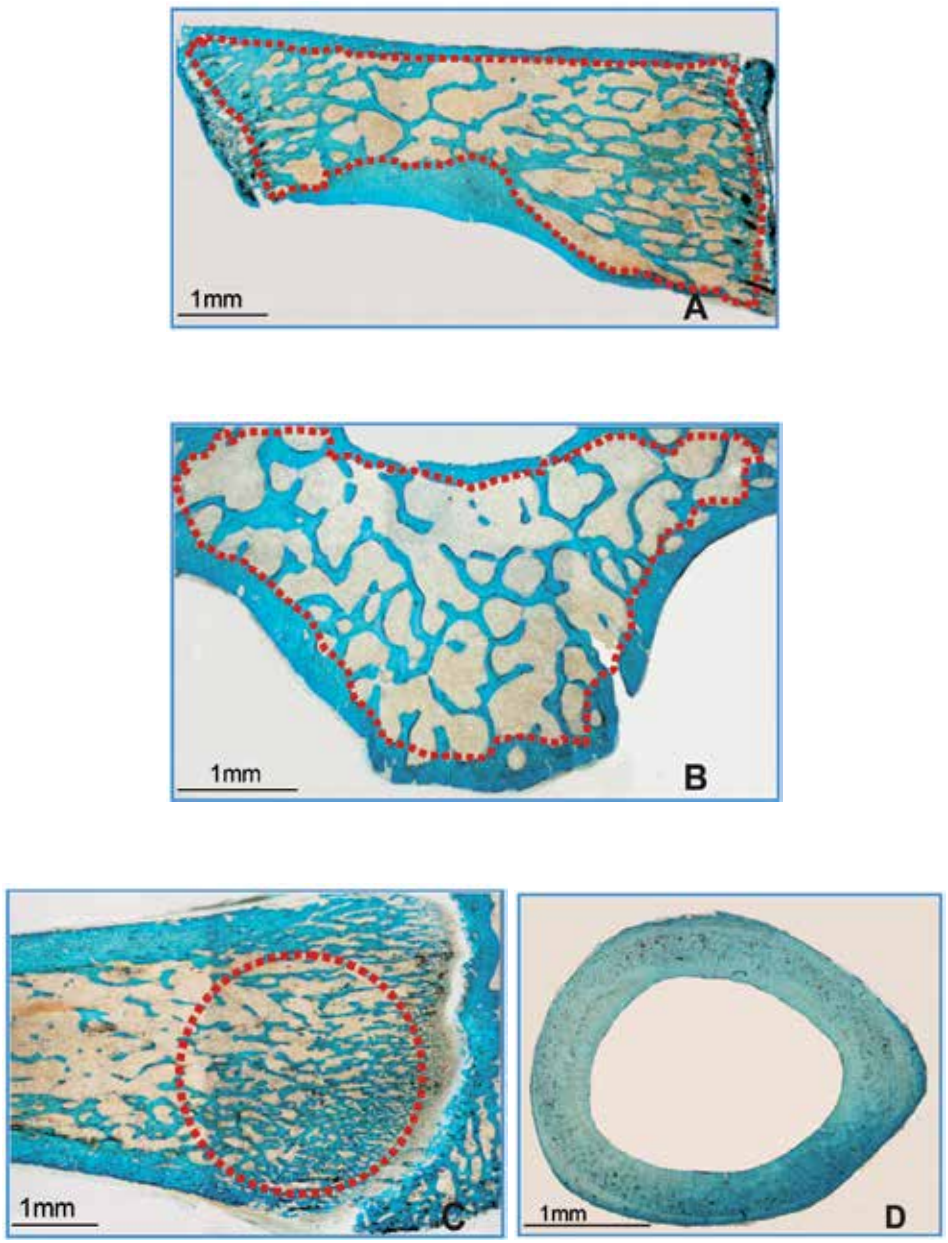


Fig. 3. Histological sections taken from SHAM group in which the histomorphometrical analyses were performed. (A) sagittal section of 4th lumbar vertebra; (B) transversal section of 5th lumbar vertebra; (C) sagittal section of the distal epiphyseal level of femur; (D) transversal section at the mid-diaphyseal level of femur. The dotted lines indicate the areas in which evaluations were recorded. (Figure by Palumbo et al., 2009).

2.3 Biochemical assays

Blood samples from experimental rats were collected in tubes and the serum was immediately separated by centrifugation, aliquoted into small volumes and stored at –20°C for analysis. The serum levels of magnesium, calcium, inorganic phosphorus and alkaline phosphatase (ALP) activity were determined by colorimetry using commercially available test kits.

2.4 Statistical analysis

One-way analysis of variance (ANOVA) with Newman-Keuls test for post-hoc comparisons between individual treatment groups and controls was performed. Student's *t*-test was used where appropriate. Values of $P<0.05$ indicate significant differences between groups.

3. Results

3.1 Body weights

Both in preventive and recovering studies, initial body weights of the four animal groups were similar.

In preventing study (Table 1), as expected, the body weight of C-OVX rats, sacrificed at 30 and 60 days after ovariectomy, was significantly higher than that of SHAM animals. The chronic administration of ferutinin as well as estradiol benzoate significantly counterbalanced body weight increase. It must be stressed that estradiol benzoate (EB) treatment was able to equal the body mass gain of sham-operated control rats, while ferutinin caused a more marked decrease in body weight in comparison to EB.

TABLE 1 - Body weight in Preventing study

Treatment group	Initial BW	BW at 30th day	BW at 60th day
SHAM	198.9±2.4	249.7±4.1	246.0±5.4
C-OVX	205.1±2.1	308.4±5.6[a]	335.2±9.8[a]
F-OVX	196.8±1.7	194.4±4.0[b,c]	246.0±5.4
EB-OVX	204.7±3.1	229.8±1.9[b]	246.8±3.4[b]

Table 1. Effect of ferutinin and estradiol benzoate on body weight of ovariectomized rats. Values represent mean±SEM. Anova followed by Newman-Keuls post test: [a]$P<0.001$ vs. SHAM, [b]$P<0.001$ vs. C-OVX, [c]$P<0.001$ vs. EB-OVX. SHAM: sham-operated controls receiving vehicle; C-OVX: ovariectomized controls receiving vehicle; F-OVX: ovariectomized treated with ferutinin; EB-OVX: ovariectomized treated with estradiol benzoate; BW: body weight.

In recovering study (Fig. 4) two months after ovariectomy (namely, at the beginning of treatment) the body weight of ovariectomized rats (C-OVX, F-OVX and EB-OVX) was significantly higher, as expected, with respect to SHAM, but after both 30 and 60 days of chronic administration of ferutinin as well as of estradiol benzoate the body weight reduces significantly in comparison to C-OVX and it is similar to that of SHAM one.

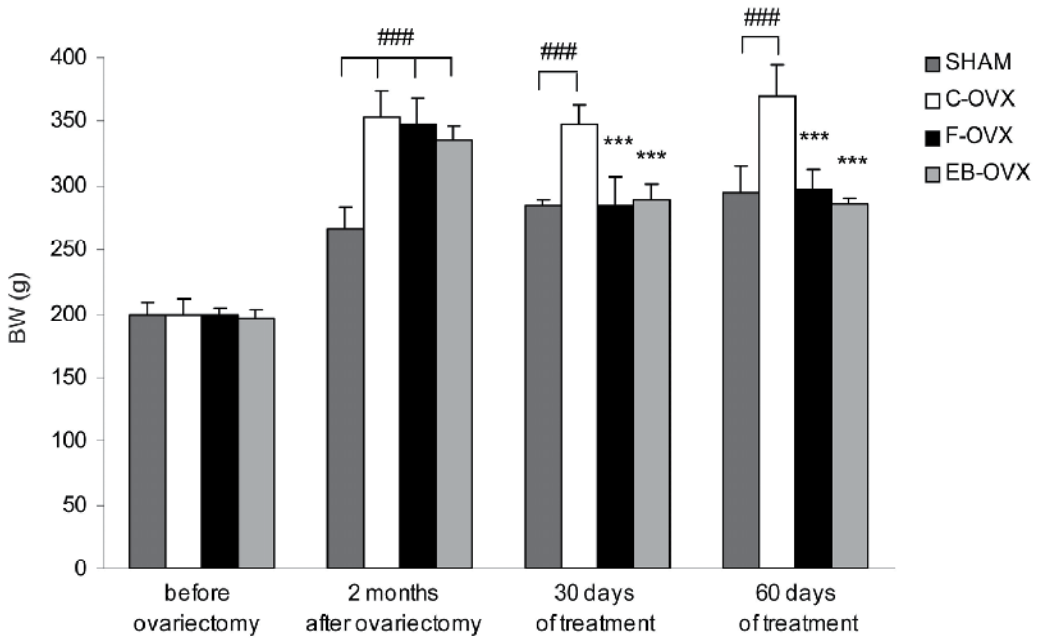


Fig. 4. Recovering study. Histograms showing the mean values of body weights-BW (g) recorded from all animal groups at 4 different times: (1) at the start of the experiment (before ovariectomy), (2) two months after ovariectomy, (3) after 30 days of treatment and (4) after 60 days of treatment. Values are expressed as mean ± SEM. $^{***}P<0.001$ vs. C-OVX; $^{###}P<0.001$ vs. SHAM (Anova followed by Newman-Keuls test). SHAM: sham-operated controls receiving vehicle; C-OVX: ovariectomized controls receiving vehicle; F-OVX: ovariectomized treated with ferutinin; EB-OVX: ovariectomized treated with estradiol benzoate; BW: body weight.

3.2 Histology and histomorphometric analysis

3.2.1 Bone mass in preventing study protocol

Histological observations of bone sections of vertebrae and femurs from treated and control animal groups underlined, as it is expected, that bone mass is clearly lower in C-OVX rats, with respect to SHAM and treated (F-OVX and EB-OVX) animals (Fig. 5).

The histomorphometric results obtained after 30 and 60 days of treatment showed that ovariectomy induced reduction in bone mass of lumbar vertebrae and femur, which is not observed in the animals treated with ferutinin or estradiol benzoate (Figs. 6 and 7); in particular, comparing the two groups of ovariectomized animals treated with ferutinin (F-OVX) and estradiol benzoate (EB-OVX), the mean values are always higher in F-OVX with respect to EB-OVX, sometimes displaying statistical significance.

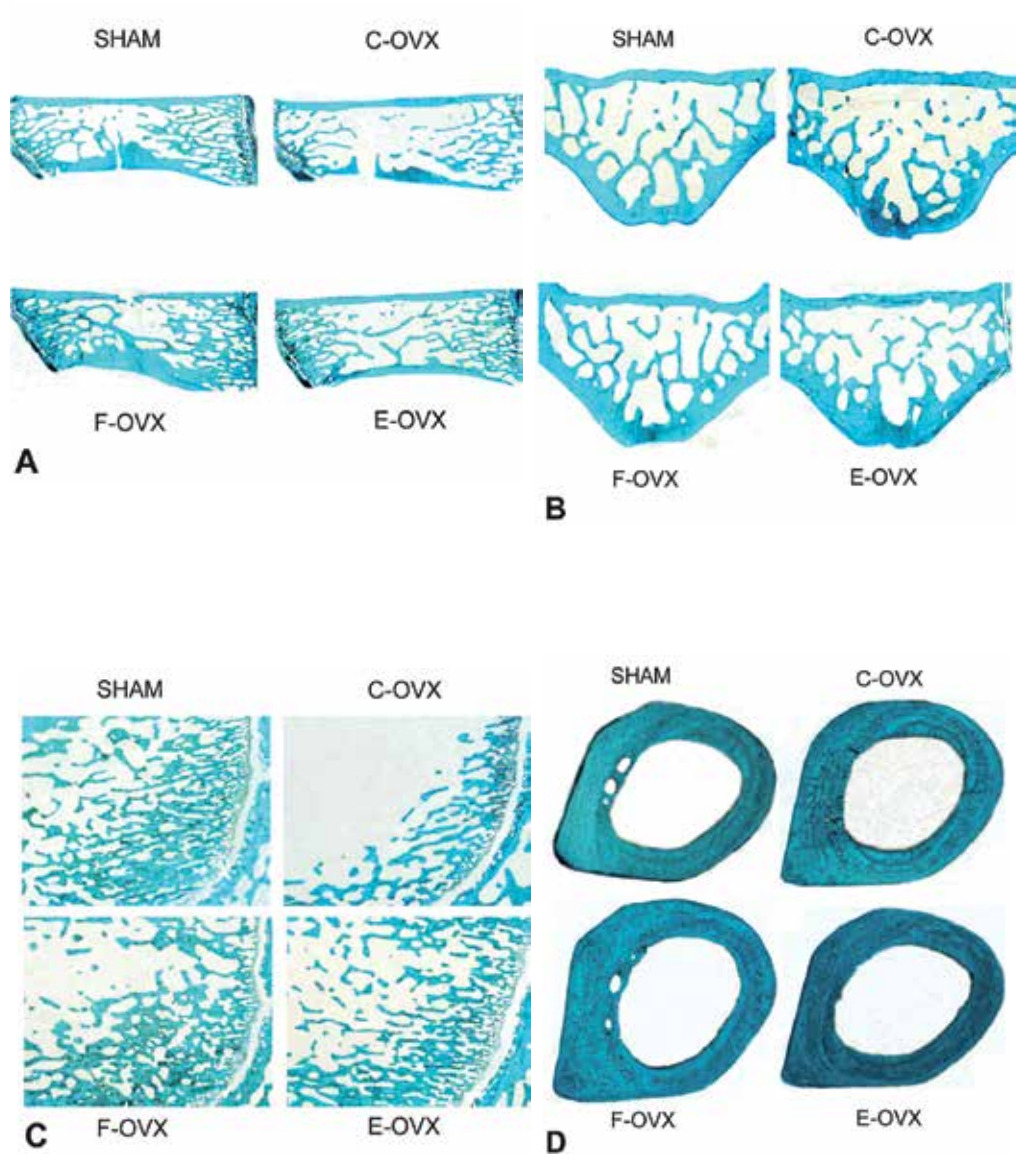


Fig. 5. Preventing study. LM micrographs showing the bone histology from the four experimental animal groups: (A) sagittal sections of 4th lumbar vertebra; (B) transversal sections of 5th lumbar vertebra; (C) sagittal sections of the distal epiphyseal level of femur; (D) transversal sections at the mid-diaphyseal level of femur. (Figure by Palumbo et al., 2009).

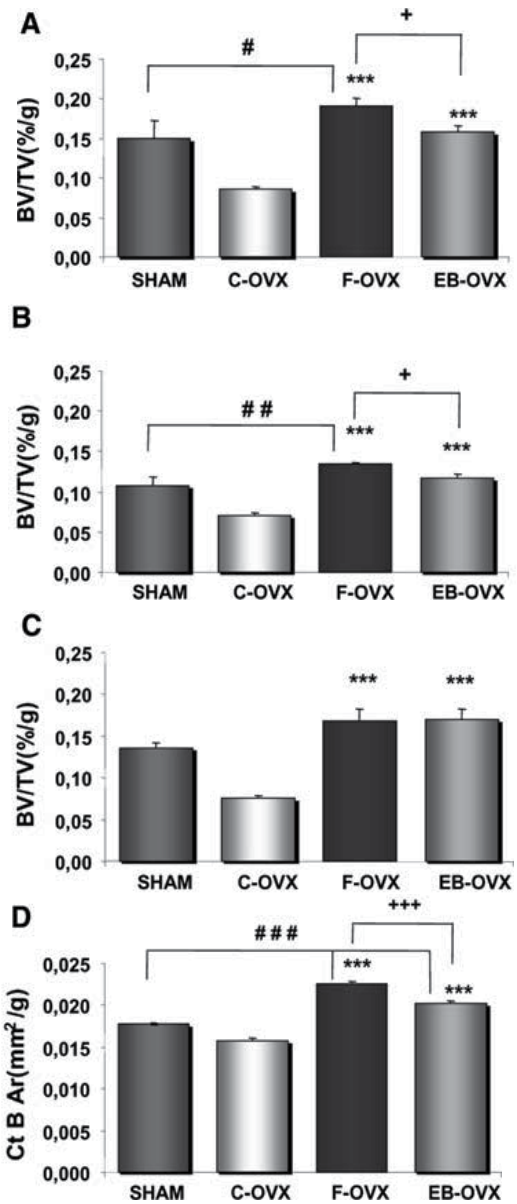


Fig. 6. Preventing study. Mean values of histomorphometric normalized parameters, expressed as BV/TV (%/g) and CT-B-Ar (mm^2/g), in both trabecular and cortical bone of the four animal groups, after 30 days from ovariectomy. (A) sagittal sections of 4th lumbar vertebra; (B) transversal sections of 5th lumbar vertebra; (C) sagittal sections of the distal epiphyseal level of femur; (D) transversal sections at the mid-diaphyseal level of femur. Values are expressed as mean ± SEM. ***P<0.001 vs. C-OVX; ^{+++}P<0.01 vs. EB-OVX; $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 vs. SHAM (ANOVA followed by Newman–Keuls test). SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.

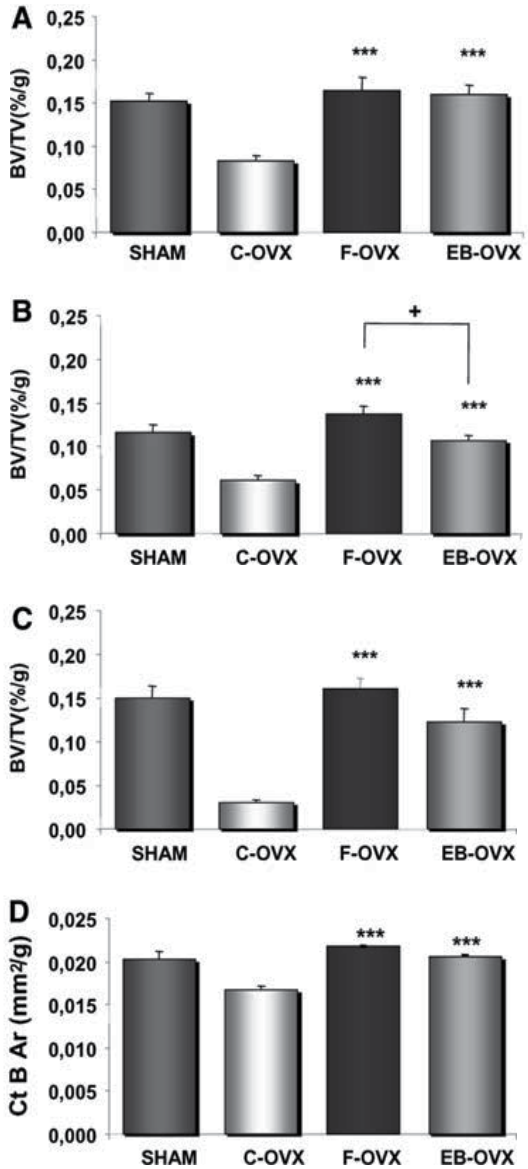


Fig. 7. Preventing study. Mean values of histomorphometric normalized parameters, expressed as BV/TV (%/g) and Ct-B-Ar (mm^2/g), in both trabecular and cortical bone of the four animal groups, after 60 days from ovariectomy. (A) Sagittal section of 4^{th} lumbar vertebra; (B) transversal section of 5^{th} lumbar vertebra; (C) sagittal section of the distal epiphyseal level of femur; (D) transversal section at the mid-diaphyseal level of femur. Values are expressed as mean ± SEM. ***P<0.001 vs. C-OVX; $^{+}$P<0.05 vs. EB-OVX (ANOVA followed by Newman–Keuls test). SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin, EB-OVX ovariectomized treated with estradiol benzoate.

3.2.2 Bone mass in recovering study protocol

As regards both periods of time (30 and 60 days), the histological sections of vertebrae and femurs from SHAM and treated animal groups showed higher amount of trabecular bone (Figure 8A-B-C) with respect to C-OVX group, while the amount of cortical bone did not show differences among all groups (Fig. 8D). The histomorphometric analyses clearly showed the different results in trabecular and cortical bone: the amount of trabecular bone (Figures 9A-B-C and 10A-B-C) in F-OVX and EB-OVX animals are always higher with respect to C-OVX ones, although they do not reach the values of SHAM animals; as far as cortical bone (Figures 9D and 10D) is concerned, no statistically significant differences were found in bone area among all groups after both 30 and 60 days of treatments.

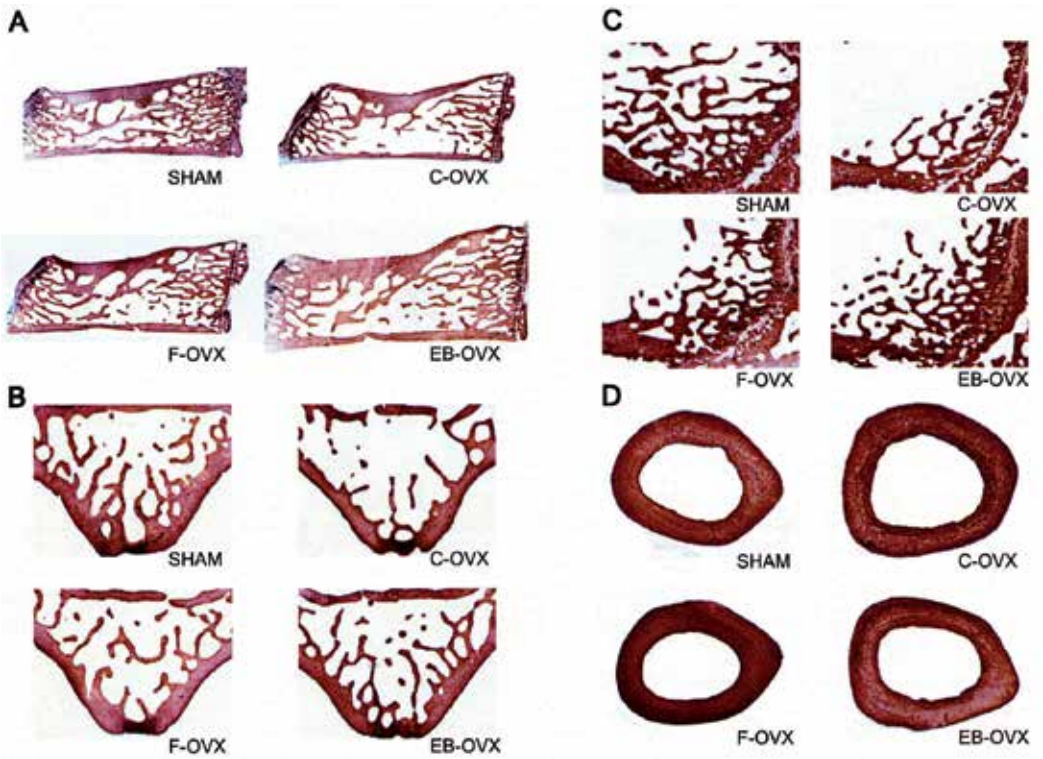


Fig. 8. Recovering study. LM micrographs showing the bone histology from the four experimental animal groups after 30 days of treatment. (A) Sagittal sections of the 4th lumbar vertebra; (B) transversal sections of the 5th lumbar vertebra; (C) sagittal sections of the distal epiphysis of femur; (D) transversal sections at the mid-diaphyseal level of femur. (Figure by Ferretti et al., 2010).

3.2.3 Uterine tissues

Preliminary data, not yet published by the authors, concern also the side effects of the chronic treatment with ferutinin on the uterus of ovariectomized rats, particularly regarding weight, size, morphology and structure. The target was to compare ferutinin side effects with those elicited by estradiol benzoate treatment, both in preventing and recovering protocols. Although data are incomplete, ferutinin would seem to exert the same effect of

estrogen benzoate in increasing uterine weight not only in the preventing study but also in the recovering one. In particular, the morphological and morphometrical preliminary data suggest that ferutinin would act on the uterus in a manner similar to that of estradiol benzoate, stimulating endometrial hypertrophy. Moreover, the treatment with ferutinin is of particular interest because the apoptotic index in both preventing and recovering studies seems to be almost always higher in both luminal and glandular endometrial epithelia with respect to animal groups treated with estradiol benzoate.

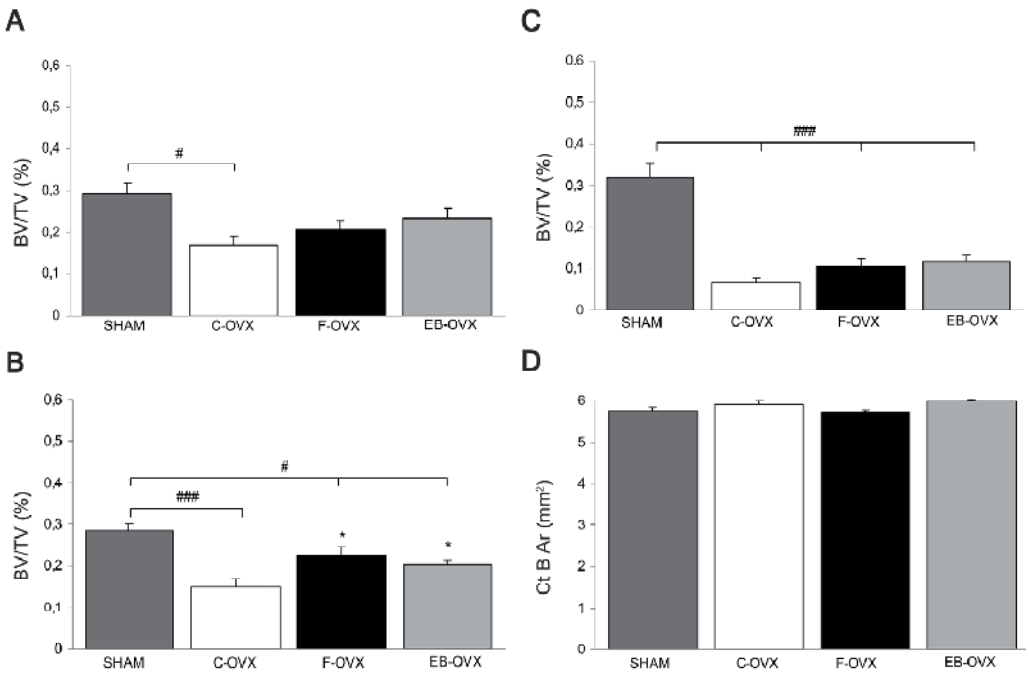


Fig. 9. Recovering study. Mean values of histomorphometric parameters, expressed as BV/TV (%) and Ct-B-Ar (mm^2), in both trabecular and cortical bone of the all animal groups after 30 days of treatment. (A) Sagittal section of the 4th lumbar vertebra; (B) transversal section of the 5th lumbar vertebra; (C) sagittal section of the distal epiphysis of femur; (D) transversal section at the mid-diaphyseal level of femur. Values are expressed as mean ± SEM. $^{*}P < 0.05$ vs. C-OVX; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. SHAM (ANOVA followed by Newman–Keuls test). SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.

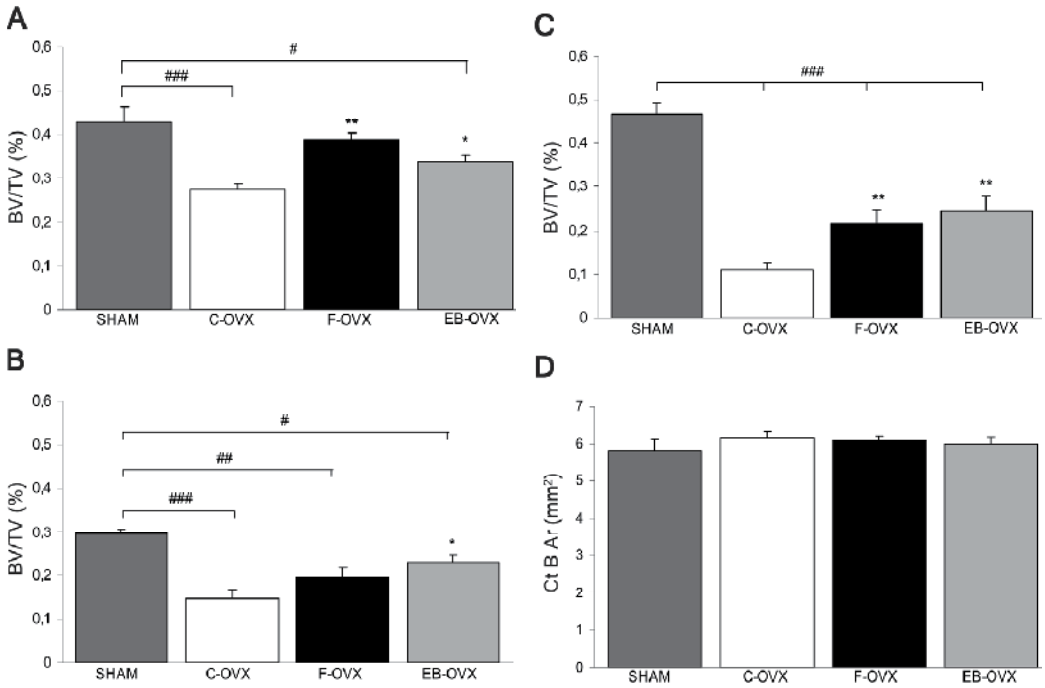


Fig. 10. Recovering study. Mean values of histomorphometric parameters, expressed as BV/TV (%) and Ct-B-Ar (mm^2), in both trabecular and cortical bone of the all animal groups after 60 days of treatment. (A) Sagittal section of the 4th lumbar vertebra; (B) transversal section of the 5th lumbar vertebra; (C) sagittal section of the distal epiphysis of femur; (D) transversal section at the mid-diaphyseal level of femur. Values are expressed as mean ± SEM. *P< 0.05, **P< 0.01 vs. C-OVX; #P< 0.05, ##P< 0.01, ###P< 0.001 vs. SHAM (ANOVA followed by Newman–Keuls test). SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.

3.3 Serum biochemical analysis

In preventing study protocol, serum level variations of magnesium, calcium and inorganic phosphorous among the groups were more evident after 30 days of treatment rather than after 60 days (Table 2), while in the recovering study protocol, no significant differences in serum levels were recorded (Table 3). As far as serum alkaline phosphatase is concerned, its levels in F-OVX animal groups were always higher with respect to all other groups both in preventive and recovering study protocols after 30 as well as 60 days of treatment.

TABLE 2	Treatment group	Mg (mg/dl)	Ca (mg/dl)	P inorganic (mg/dl)	ALP (UI/l)
30 day-treatment	SHAM	2.82±0.02	9.8±0.1	9.06±0.22	110.3±12.0
	C-OVX	2.94±0.09	10.2±0.05$^{\#}$	9.85±0.35	106.2±4.9
	F-OVX	2.74±0.04	9.6±0.1$^{**\ ++}$	7.59±0.21$^{***\ \#\#\ ++}$	148.4±17.5
	EB-OVX	2.88±0.05	10.1±0.07	9.07±0.26	111.0±8.4
60 day-treatment	SHAM	2.54±0.01	9.7±0.1	7.81±0.17	90.8±5.1
	C-OVX	2.52±0.05	9.7±0.2	7.76±0.34	102.6±4.2
	F-OVX	2.55±0.05	9.9±0.1	7.05±0.31	118.0±7.1^{++}
	EB-OVX	2.53±0.05	9.7±0.2	7.03±0.33	79.7±7.3

Table 2. Preventing study. Effect of ferutinin/estradiol benzoate on serum biochemical values of ovariectomized rats treated for 30 and 60 days. All values are expressed as mean ± SEM. Anova followed by Newman-Keuls post test: $^{**}P<0.01$, $^{***}P<0.001$ vs. C-OVX; $^{++}P<0.01$ vs. EB-OVX; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. SHAM. SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.

TABLE 3	Treatment group	Mg (mg/dl)	Ca (mg/dl)	P inorganic (mg/dl)	ALP (UI/l)
30 day-treatment	SHAM	2.43±0.06	10.6±0.01	6.29±0.43	103±13.65
	C-OVX	2.41±0.07	10.18±0.18	7.18±0.3	81.6±3.98
	F-OVX	2.43±0.04	10.56±0.1	6.09±0.22	144.6±15.4$^{**\ \#\ ++}$
	EB-OVX	2.48±0.05	10.52±0.1	6.57±0.25	75±12.5
60 day-treatment	SHAM	2.57±0.06	10.25±0.16	7.52±0.46	89.5±9.24
	C-OVX	2.86±0.4	10.28±0.02	6.2±0.22$^{\#}$	90.2±8.61
	F-OVX	2.55±0.07	10.58±0.12	6.37±0.27$^{\#}$	109.2±7.19^{+}
	EB-OVX	2.47±0.04	10.58±0.14	6.02±0.17$^{\#}$	72.8±5.91

Table 3. Recovering study. Effect of ferutinin/estradiol benzoate on serum biochemical values of ovariectomized rats (30 and 60 days of treatment). All values are expressed as mean ± SEM. Anova followed by Newman-Keuls post test: $^{**}P<0.01$ vs. C-OVX; $^{+}P<0.05$, $^{++}P<0.01$ vs. EB-OVX; $^{\#}P<0.05$ vs. SHAM. SHAM sham-operated controls receiving vehicle; C-OVX ovariectomized controls receiving vehicle; F-OVX ovariectomized treated with ferutinin; EB-OVX ovariectomized treated with estradiol benzoate.

4. Discussion

The results so far obtained have clearly suggested that ferutinin displays positive effects on bone mass both in preventing and in curative treatment of estrogen deficiency osteoporosis; more precisely, the observations have indicated that ferutinin seems to exert similar effects to estradiol benzoate in curative treatment (Ferretti et al., 2010), and even it seems to be

more effective, compared with estradiol benzoate, in preventing bone loss due to estrogen deficiency (Palumbo et al., 2009). It is to be underlined that, comparing the results from the two protocols, in curative study the values of bone mass of treated animals never reach those of SHAM group; this is due to the fact that the treatment started after the occurrence of a severe osteoporosis (as a consequence of estrogen deficiency secondary to two months of ovariectomy).

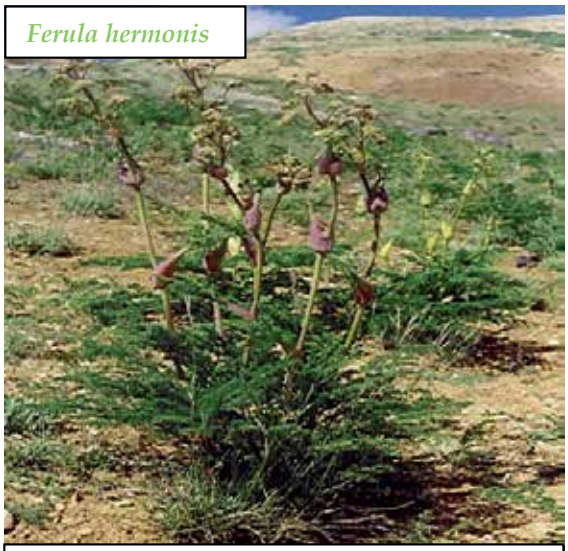
It is important to emphasise that one of the most important effects of skeletal diseases, like osteoporosis, is the progressive trabecular bone resorption that, in turn, implies enhanced bone fragility and, consequently, an increased frequency of fractures. According to the literature (Kalu, 1991; Wronski et al., 1987) ovariectomy in rats induces different effects on trabecular bone of the axial with respect to the appendicular skeleton, with more marked bone resorption taking place in the latter; for this reason, both vertebrae and femurs were investigated. Moreover, ovariectomy differently affects trabecular and cortical bone, since in OVX animals the bone mass loss observed in trabecular bone was not equally observed in cortical bone; in fact, the values of femur cortical bone areas are similar in OVX and SHAM groups. These data are in line with older ones showing an earlier started decrease in bone mass, more extensive in the spongiosa than in the compacta of rats fed a low-calcium diet (Lozupone & Favia, 1988). This fact is a consequence of the different pattern of distribution of mechanical stresses acting on the two different bony architectures and it is probably related to the different metabolism of the various skeletal regions that, in turn, affect the bone turnover rate of the different skeletal regions, viz. metaphysis compared with diaphyses (Canè et al., 1982). Other authors have also shown that cortical bone is not very sensitive to bone loss due to ovariectomy standing the increased endosteal osteoblasts (Jee et al., 1990; Liu & Kalu, 1990; Turner et al., 1987). All these considerations make the "Bone Organ" a sophisticated system in which metabolic and mechanical demands are actually sensed and integrated in answering both systemic and loading needs.

As previously mentioned the authors wanted to evaluate whether the chronic administration of ferutinin, starting from the day after ovariectomy, is able to prevent estrogen deficiency effects similarly to HRT. The results obtained clearly showed that the phytoestrogen ferutinin displays positive effects in preventing osteoporosis due to estrogen deficiency; more precisely the observations suggest that ferutinin seems to be more effective in preventing bone loss compared with estradiol benzoate. Another positive aspect of ferutinin treatment is to prevent weight gain that typically occurs after ovariectomy. As above mentioned, ferutinin has been shown to interact with estrogen receptors (Appendino et al., 2002; Ikeda et al., 2002). While the majority of phytoestrogens have a higher relative binding affinity for ERβ than ERα, ferutinin displays a higher affinity for ERα (IC_{50}=33.1 nM) than for ERβ (IC_{50}=180.5 nM) (Ikeda et al., 2002). The different roles of specific estrogen receptors ERα and ERβ on body weight regulation were recently investigated by Wegorzewska and co-workers (2008), using the ovariectomized rat model. OVX rats showed a significant increase in body weight, which was reversed by the daily treatment (for 21 days) with estradiol or PPT (propylpyrazoletriol, a selective ERα agonist), but not by the daily treatment with DPN (diarylpropionitrile, a ERβ agonist); these results confirm the major role of ERα in regulating body weight, as it was previously suggested by other authors (Kraichely et al., 2000; Stauffer et al., 2000) by using ER-specific knockout mice.

Regarding the bone turnover-related serum levels, the recorded values of alkaline phosphatase (the most widely recognized biochemical marker for osteoblastic activity -

Evans et al., 1990; Nian et al., 2006) suggest that the process of osteogenesis should be triggered in F-OVX group, because ALP value in F-OVX is higher with respect to the other groups. A positive effect on osteoblast activity *in vitro* by other phytoestrogens, like genistein, has already been published (Liao et al., 2007; Pan et al., 2005).
As far as ferutinin side effects is concerned on the organs commonly targeted by estrogens, the apparent above cited antiapoptotic effect on endometrial epithelia is in line with observations previously recorded for genistein by other authors that administered such phytoestrogen to ovariectomized mice (Eason et al., 2005; Garcìa-Pérez et al., 2006). Since an increased risk of endometrial cancer due to excessive hypertrophy is one of the recognized prejudicial effects of estrogens, the phytoestrogen ferutinin, althought induces thickening of endometrium as well as estrogens, seems to increase the percentage of apoptotic epithelial cells, particularly the glandular ones. This effect might exert a protective role against uterine carcinoma.

Ferula hermonis



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5. Conclusion

On the light of the observations above reported on the effect of ferutinin in preventing/recovering severe osteoporosis secondary to ovariectomy in rats, the authors suggest to enumerate ferutinin among the osteoprotective substances. This fact acquires a more relevant importance in the light of recent tenable evidences, as above cited, reported from some authors concerning the absence of negative side effects by some phytoestrogens (particularly genistein, 8-prenylnaringenin, reveratrol and red clover extract) on the tropism of various organs commonly targeted by estrogens (Burdette et al., 2002; Duffy et al., 2007; Eason et al., 2005; Gallo et al., 2006; Garcia-Perez et al., 2006; Hümpel et al., 2005; Lian et al., 2001; Limer & Speirs, 2004; Murray et al., 2003; Whitsett & Lamartiniere, 2006; Wu et al., 2002).

In conclusion, the results here reported not only provide evidence that ferutinin can significantly prevent/recover ovariectomy-induced bone loss in rats, but also that it could protect against the onset of uterus cancer. Although the putative undesired estrogenic-like side effects on uterus of such phytoestrogen have not yet been fully investigated, ferutinin could be an interesting safer alternative new candidate for HRT in treatment of post-menopausal symptoms, since it seems to protect from bone loss induced by ovariectomy (Ferretti et al., 2010; Palumbo et al., 2009) and in part to mime the ovarian endocrine function during menopause. The authors are aware that additional studies are required to characterize the mechanism by which ferutinin acts both in improving/resolving severe degrees of bone mass loss and in protecting from uterine cancer onset.

6. Acknowledgments

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36

The Phytoestrogens, Calcitonin and Thyroid Hormones: Effects on Bone Tissue

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1. Introduction

The skeleton is a metabolically active organ that undergoes remodeling throughout life. This involves a complex process by which old bone is continuously replaced by new tissue. Bone remodeling refers to the sequential, coupled actions of osteoclasts and osteoblasts. In conditions of sex hormone deficiency during advancing age, after the menopause or andropause, the rate of remodeling increases and bone formation is reduced relative to resorption. These alterations can cause microarchitectural deterioration of bone tissues, which increases bone loss as a predisposition to the occurrence of osteoporosis (Rehman et al., 2005). However, in contrast to postmenopausal osteoporosis in women, age-related bone loss in men is less well defined.

Numerous studies attest to the importance of estrogen in bone remodeling, evident from the finding that hormone replacement therapy (HRT) administered in a dose-dependent manner effectively prevented bone loss in postmenopausal women (Lindsay et al., 1976, 1984). However, in addition to protective effects on bone, HRT is associated with an increased risk for breast, endometrial, ovarian or prostate cancers (Davison & Davis, 2003; Loughlin & Richie, 1997; Nelson et al., 2002). Therefore, it is important to examine alternative approaches for prevention and treatment of osteoporosis without side effects. It is well known that the incidence of osteoporosis-related fractures is significantly lower in Southern and Eastern Asian women than in Western women (Tham et al., 1998). One possible reason for this difference is a high intake of phytoestrogen-rich plants, which Asian people eat more often than Western people (Ho et al., 2003). As a result, over the past decade a number of clinical trials for prevention of bone loss have assessed the effectiveness of plant derived non-steroidal phytoestrogens found in a wide variety of foods, most notably soybean. Isoflavones, which include daidzein and genistein are a class of phytoestrogens that act like estrogens. Since these compounds bind to estrogen receptors (ERs) and have estrogen-like activity (Branca, 2003), they have attracted much attention because of their potential benefit in the prevention and treatment of osteoporosis.

In addition to the phytoestrogen-mediated protective mechanisms against bone loss, recent evidence suggests that daidzein may also act on rat bone tissue through enhancement of thyroid C cell activity (Filipović et al., 2010). Namely, thyroid C cells produce the hormone, calcitonin (CT), which lowers plasma calcium concentration by suppressing osteoclast activity. Synthesis of CT and its release from C cells were decreased in conditions of gonadal hormone

deficiency (Filipović et al., 2003, 2007; Isaia et al., 1989; Lu et al., 2000; Sakai et al., 2000). Due to its osteoprotective properties, CT is widely applied in the therapy of osteoporosis.
It is known that parathyroid hormone (PTH) is a major factor involved in the systemic regulation of bone resorption. Phytoestrogens may affect the parathyroid gland and reduce PTH secretion (Wong et al., 2002), suggesting that one way in which these compounds inhibit bone loss may be through reducing PTH levels.
Thyroid hormones are essential for normal bone maturation *in utero* and during early life. In adults an excess of thyroid hormones in the body affects the remodeling system in cortical and trabecular bone and may contribute to the development of osteoporosis (Kung, 1994). Receptors for these hormones are present in bone cells and they may directly increase bone resorption (Abu et al., 1997; Rizzoli et al., 1986). Additionally, thyroid-stimulating hormone (TSH), which stimulates the release of thyroid hormones, positively influences bone remodeling. Therefore, demonstrating both anabolic and antiresorptive effects, TSH may represent a promising candidate for the treatment of osteoporosis (Sendak et al., 2007).
In this chapter we will describe the known effects of phytoestrogens on bone. In addition to the direct action of these plant compounds, special attention will be paid to their influence on thyroid C and follicular cells, as producers of CT and thyroid hormones, using the latest data in the literature and our own results. These hormones, together with PTH may be involved in the indirect effects of phytoestrogens on bone tissue.

2. Bone cells and bone remodeling

Bone is a dynamic organ that undergoes remodeling throughout life. This process results from the separate action of bone forming cells called osteoblasts and bone resorbing cells called osteoclasts. Osteoblasts are responsible for the production of bone matrix constituents and are found in clusters on bone surfaces (Fig 1). They originate from multipotent mesenchymal stem cells, which have the capacity to differentiate into osteoblasts or other cells, such as adipocytes, chondrocytes, myoblasts and fibroblasts (Bianco et al., 2001). A mature osteoblast that is trapped in the bone matrix and remains isolated in lacunae becomes an osteocyte. (Fig.1). Bone formation involves production and maturation of the osteoid matrix, followed by mineralization of the matrix. Osteoblasts produce growth factors, such as insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF- β) and bone morphometric protein (BMP) (Canalis et al., 1993, 1993a; Chen et al., 2004; Globus et al., 1989; Rydzel et al., 1994). These factors regulate osteoblast activity in an autocrine and paracrine manner.
Osteoclasts are large multinucleate cells responsible for bone resorption. They are derived from hematopoetic cells of the mononuclear lineage (Teitelbaum, 2000) (Fig.1). Osteoclasts have an abundant Golgi complex, mitochondria and transport vesicles loaded with lysosomal enzymes, such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K. These enzymes are secreted via the specialized (ruffler border) plasma membrane of osteoclasts into the bone-resorbing compartment (Väänänen et al., 2000). The process of osteoclast attachment to the bone is complex and involves binding of integrins expressed in osteoclasts with specific amino acid sequences within proteins at the surface of the bone matrix and cytoskeleton activation (Davies et al., 1989; Reinholt et al., 1990). Dynamic structures, called podozomes allow movement of osteoclasts across the bone surface. Bone resorption occurs due to acidification and proteolysis of the bone matrix. As a result of this resorptive activity in contact

with the surface of calcified bone, osteoclasts create resorptive lacunae. Osteoclast function is regulated both by locally acting cytokines and by systemic hormones.

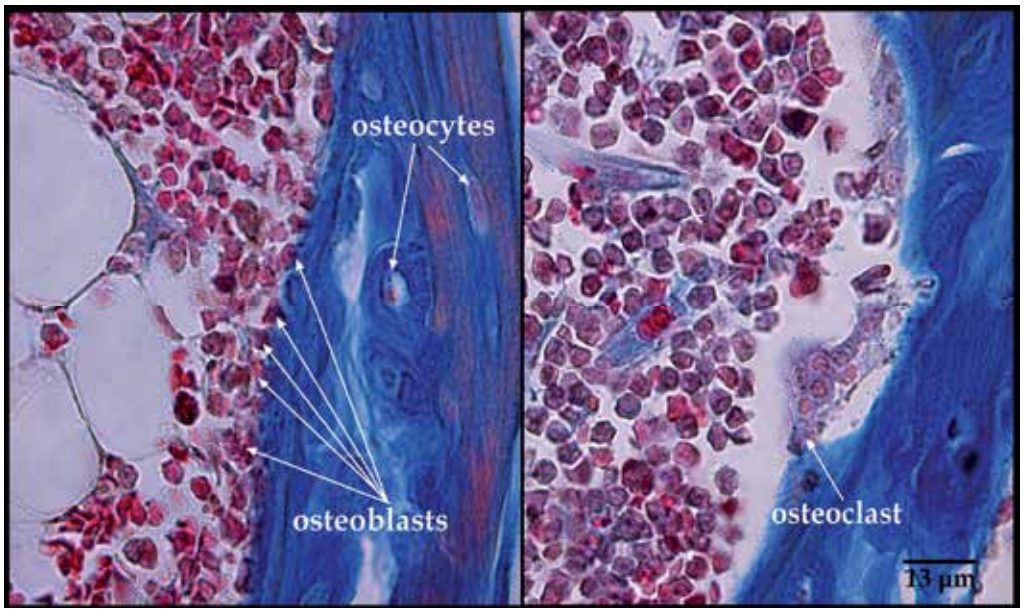

Fig. 1. Bone cells – osteoblasts, osteocytes and osteoclasts; unpublished image of Filipović et al.

In homeostatic equilibrium, bone resorption and formation are balanced. It appears that osteoclasts and osteoblasts closely collaborate in the remodeling process in what is called a "Basic Multicellular Unit", or BMU. This indicates that a coupling mechanism must exist between formation and resorption (Frost, 1964), although its nature is not known. Organization of the BMU in cortical and trabecular bone differs. Between 2% and 5% of cortical bone is remodeled each year. The remodeling process in trabecular bone is mainly a surface event. Due to the much larger surface to volume ratio, it is more actively remodeled than cortical bone, with remodeling rates that can be up to 10 times higher (Lee & Einhorn, 2001).

The remodeling cycle consists of three consecutive phases: resorption, reversal and formation. Resorption begins with the migration of partially differentiated preosteoclasts, which form multinucleated osteoclasts on the bone surface. During the reversal phase, mononuclear cells prepare the resorption lacunae for bone formation and provide signals for osteoblast differentiation and migration (Eriksen et al., 1990). Bone formation starts with activation of preosteoblasts to differentiate into osteoblasts. They secrete bone-matrix proteins to form the organic matrix, which is later mineralized. During this period, osteoblasts completely replace the resorbed bone by new tissue. After this phase, the surface is covered with flattened lining cells and a prolonged resting period ensues until a new remodeling cycle is initiated. Duration of the resorption phase is about 2 weeks, the reversal phase lasts for up to 4 or 5 weeks, while the formation phase can continue for 4 months.

At each remodeling site, bone resorption is coupled with bone formation, locally released growth factors and cytokines acting as mediators of this process (Canalis et al., 1988; Mundy, 1995). The decrease of bone mass, which may be due to different causes, is a

consequence of an imbalance between the amount of mineral and matrix removed and that subsequently incorporated into each resorption cavity (Kanis et al., 1990).

3. Phytoestrogens in bone protection

Phytoestrogens are structurally and functionally similar to estrogens and their estrogenic activity may occur through ERs. There are three main classes of phytoestrogens: isoflavonoids, coumestans and lignans (Fig. 2). Due to their estrogenic and anti-estrogenic activity, they are termed - natural selective ER modulators (SERMs). Therefore, soybean isoflavones have received great attention as alternatives to HRT for the prevention of postmenopausal osteoporosis. Genistein and daidzein, the main isoflavones in soybean, may protect against osteoporosis, because they can affect both types of bone cells.

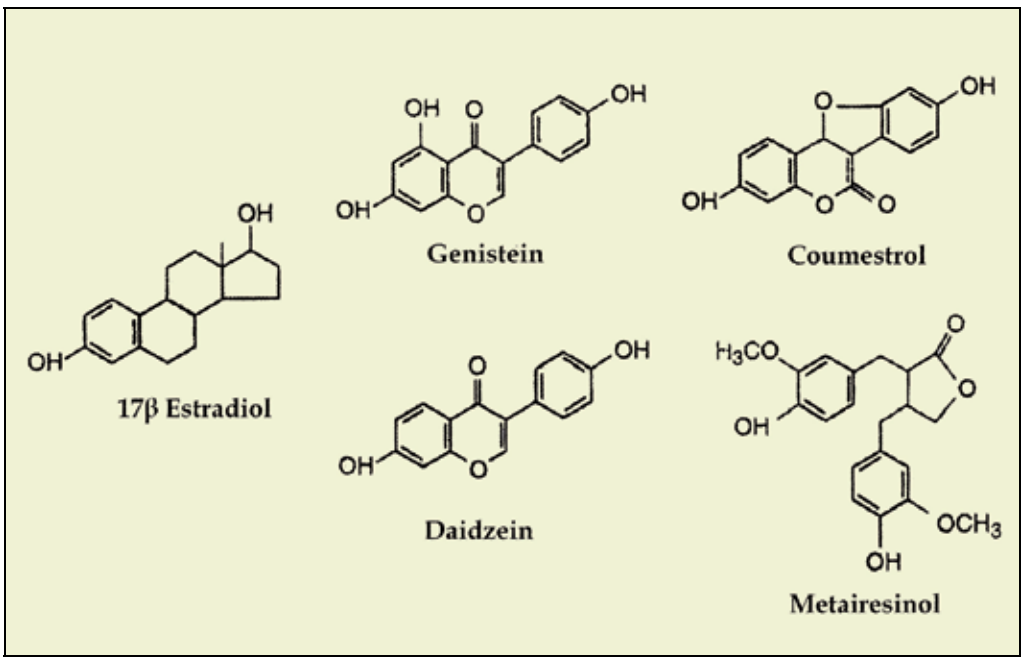

Fig. 2. Structure of 17β estradiol, isoflavones (genistein and daidzein), coumestan (coumestrol) and lignans (metairesinol); Filipović et al.

Isoflavones can stimulate the proliferation and differentiation of osteoblasts. Thus, the presence of genistein or daidzein led to a significant increase in protein synthesis, alkaline phosphatase activity, and DNA content in cultures of osteoblastic MC3T3-E1 cells (Sugimoto & Yamaguchi, 2000, 2000a; Yamaguchi & Sugimoto, 2000).

In addition to a stimulating effect on bone formation, these plant compounds may also suppress osteoclastic bone resorption in vitro. Thus, genistein was found to induce apoptosis of osteoclasts isolated from rat femoral tissues. Daidzein also decreased the number of these bone resorbing cells in rats (Gao & Yamaguchi, 1999) and their development in cultures of porcine bone marrow (Rassi et al., 2002). Osteoclast activity is regulated by phosphorylation of cell membrane constituents, involving tyrosine kinases. As a naturally tyrosine kinase inhibitor, genistein was found to suppress avian osteoclastic

activity through inhibition of tyrosine kinase (Blair et al., 1996). Genistein also caused a significant increase in tyrosine phosphatase activity, which is a negative regulator of osteoclastogenesis and osteoclast-resorbing activity in mutant mice (Aoki et al., 1999; Gao &Yamaguchi, 2000) (Fig 3).

While investigations in vitro give clues about the effects of isoflavones on individual bone cells, studies in vivo provide knowledge about their influence in intact systems. Aged gonadectomized female and male rodents are suitable animal models for studying osteoporosis (Comelekoglu et al., 2007; Filipović et al., 2007; Pantelić et al., 2010; Vanderschueren et al., 1992.) Using them it has been demonstrated that isoflavones can prevent bone loss in female rats and mice after ovariectomy (Ovx) (Blum et al., 2003; Erlandsson et al., 2005; Fonseca & Ward, 2004; Ishimi et al., 1999; Lee et al., 2004; Om & Shim, 2007; Ren et al., 2007; Wu et al., 2004). The bone-preventing effects of isoflavones were also confirmed in male orchidectomized (Orx) rats and mice (Filipović et al., 2010; Ishimi et al., 2002; Khalil et al., 2005; Soung et al., 2006; Wu et al., 2003). On the contrary, some studies showed that isoflavones had minimal or no effects on bone loss in animal models (Bahr et al., 2005; Nakai et al., 2005; Picherit et al., 2001). Moreover, in the monkey, a nonhuman primate, dietary isoflavones do not effectively prevent ovariectomy-induced bone loss (Register et al., 2003). However, others suggested that soy phytoestrogens were protective against loss of bone volume (Ham et al., 2004).

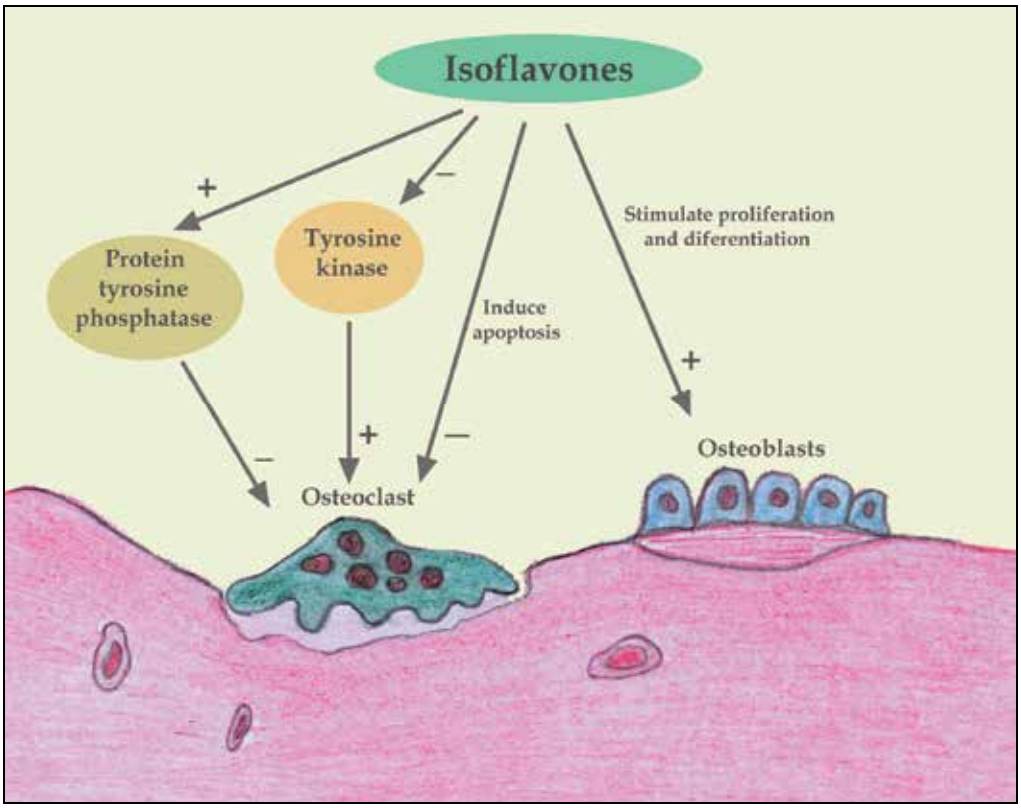

Fig. 3. Influence of isoflavones on bone cells; Filipović et al.

During recent years, numerous human studies have evaluated the effect of soy protein-containing isoflavones or pure isoflavones on bone mass. However, the results of these observational and dietary interventional investigations have been variable and conflicting. In general, isoflavone supplementation studies indicate a beneficial effect on bone mass (Huang et al., 2006; Lydeking-Olsen et al., 2004; Newton et al., 2006), no effect (Anderson et al., 2002; Arjmandi et al., 2005; Brink et al., 2008; Wu et al., 2006) or a possible negative effect in terms of increased circulating concentrations of biochemical markers associated with bone resorption (Geppert et al., 2004; Wanger et al., 2000).
The large heterogeneity of these results may be due to study design, differences regarding hormonal status of the subjects, together with the duration, type and dose of isoflavone supplementation. In addition, bone sparing benefits may depend on the extent of conversion of isoflavones to metabolites. Thus, equol binds with greater affinity to ERs than daidzein from which it is derived (Setchell et al., 2002). Equol production is dependent on the intestinal microflora and there are large interindividual differences in this metabolism. Some people produce more equol than others. Also, production of this metabolite may at least partially explain why the beneficial effects of isoflavones observed in laboratory rodents, which consistently produce high levels of equol, have not been easily recapitulated in humans, where this is not the case. Generally, the relative importance of phytoestrogens in human health must be resolved and longer-term studies are needed to determine their effects on human bone tissue.

4. Phytoestrogens – Mechanisms of action in bone

Although the mechanisms by which soy phytoestrogens may alter bone remodeling are still not completely known, Atmaca et al. (2008) state that they act on both osteoblasts and osteoclasts through genomic and nongenomic pathways.
Due to their low molecular weight these plant compounds can pass through cell membranes and interact with receptors and enzymes (Adlercreutz et al., 1998). Phytoestrogens possess estrogenic activity and act as natural SERMs. This suggests that their effect on bone can be achieved by binding to ERs. Both α and β subtypes of ERs have been identified in bone (Arts et al., 1997; Onoe et al., 1997). The protective effect of phytoestrogens is probably achieved mainly through binding to ER-β, the expression of which is increased during bone mineralization (Arts et al., 1997; Kuiper et al., 1998). In addition to ERs, phytoestrogens can bind to androgenic receptors and act as phytoandrogens (Chen & Chang, 2007).
Both genistein and daidzein stimulate osteoblast proliferation, differentiation and activation by an ER-dependent mechanism (De Wilde et al., 2004; Pan et al., 2005). These isoflavones regulated the synthesis of core binding factor-1 (Cbfa-1) and bone morphogenic protein-2 (BMP-2), which is involved in the differentiation of osteoblasts (De Wilde et al., 2004; Jia et al., 2003; Pan et al., 2005). Genistein and daidzein activate peroxisome proliferator activator receptors (PPARs). The balance between PPAR and ER activation may govern the balance between adipogenesis and osteoblastogenesis (Dang et al., 2003, 2004).
Osteoclasts express the receptor activator of nuclear factor kappa B (RANK) (Hsu et al., 1999), while the receptor activator of nuclear factor kappa B ligand (RANK-L) and osteoprotegerin (OPG) is expressed by osteoblasts (Udagawa et al., 1999). Binding of RANKL to RANK stimulates osteoclastogenesis, whereas binding of RANK-L to OPG prevents RANK-L – RANK binding and indirectly inhibits osteoclastogenesis (Fuller et al., 1998; Theoleyre et al., 2004). The relative levels this triad of proteins are important for

controlling osteoclastogenesis. It was shown that isoflavones may increase the activity of osteoblasts by stimulating the secretion of OPG and RANK-L (De Wilde et al., 2004; Yamagishi et al., 2001) (Fig. 4).

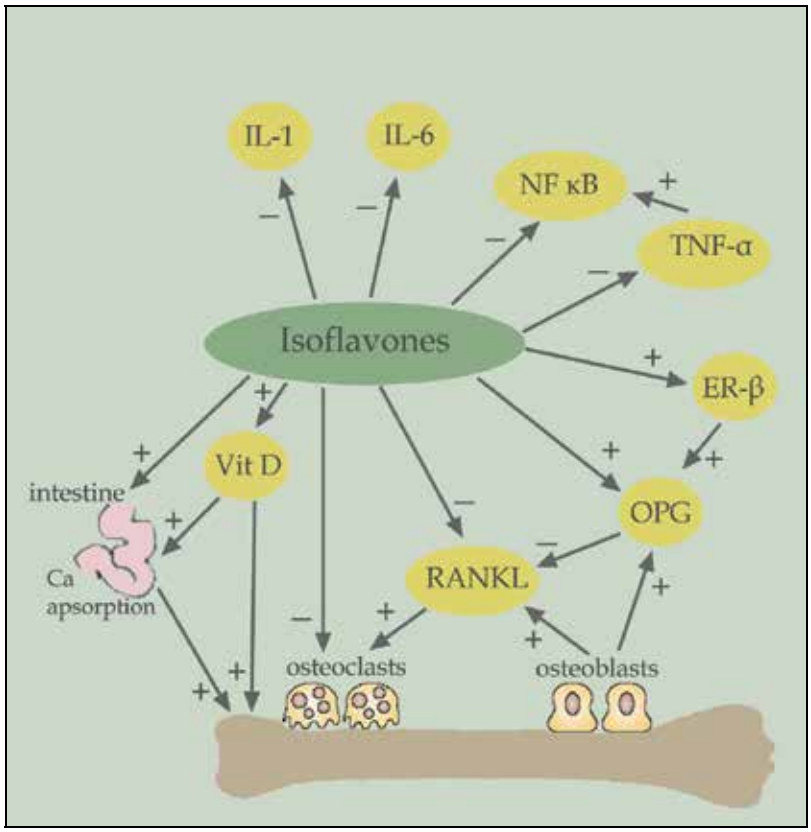

Fig. 4. Mechanisms of isoflavone action in bone; Filipović et al.

Proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α), stimulate osteoclastogenesis and bone resorption. These effects can be achieved by both RANK-L dependent and RANK-L-independent mechanisms (Collin-Osbody et al., 2001; Katagiri et al., 2002). Isoflavones have been shown to inhibit IL-6 synthesis by MC3T3-E1/4 osteoblast-like cells in vitro (Chen et al., 2003; Suh et al., 2003) and to reduce serum IL-1β and TNF-α concentrations in Ovx rats (Li, 2003). Also, a soy supplemented diet may inhibit serum concentrations of proinflammatory cytokines in postmenopausal women (Huang et al., 2005).
In addition to osteoclastogenesis, isoflavones appear to influence osteoclast activity through inhibition of inward rectifier K+channels in osteoclasts. This leads to membrane depolarization, intracellular influx of Ca2+ and inhibition of bone resorption (Okamoto et al., 2001). One beneficial effect of isoflavones on bone is increased intestinal calcium absorption (Fig. 4). However, it is not known whether the mechanism(s) by which isoflavones influence calcium absorption include interactions with intestinal ER and/or vitamin D receptor-mediated calcium transport or not (Arjmandi et al., 2002).

Nongenomic effects do not involve ERs. These effects of phytoestrogens include inhibition of tyrosine kinase which directly modulate osteoclastic acid secretion (Blair et al., 1996; Williams et al., 1998) or topoisomerase I and II, which helps to regulate cell differentiation and the cell replication cycle (Okura et al., 1998; Yamagishi et al., 2001).

5. Indirect effects of phytoestrogens on bone – The role of calcitonin, parathyroid and thyroid hormones

5.1 Effects of phytoestrogens on thyroid C cells and calcitonin production

Thyroid C cells are dispersed neuroendocrine cells that produce many bioregulatory peptides, among which CT is considered the most important. This calcium regulating hormone lowers plasma calcium concentration by inhibiting osteoclast activity. In addition to sex steroids, a voluminous literature has accumulated for therapeutic use of CT in treating osteoporosis. Thus, C cells may also be very important in the pathogenesis of osteoporosis.

The C cells are mostly located in the middle of the thyroid lobes and appear in clusters or as solitary cells between follicular cells and the capillary wall. They have a round, elliptical or polygonal shape and never face the follicular lumen. The nucleus is located in the center of the cell. The most salient ultrastructural feature of C cells is the numerous round secretory granules that fill extensive areas of the cytoplasm. The Golgi complex and endoplasmic reticulum are well developed. There is a moderate number of mitochondria, which are mostly round to elongate in shape and not uniformly distributed. Lysosomes are large and contain acid phosphatase and other lysosomal enzymes (Fig. 5).

CT suppresses the number and motility of osteoclasts (Gao & Yamaguchi, 1999; Zaidi et al., 1990) and induces a change in their contractile elements (Hunter et al., 1989). Also, CT increases osteoblast proliferation by acting on components of the insulin-like growth factor system (Farley et al., 2000) and enhancing alkaline phosphatase activity, which is associated with increased synthesis and deposition of bone matrix collagen (Farley et al., 1988, 1992; Ito et al., 1987). The action of CT bone formation is at least in part, mediated via CT receptors located on osteoblasts, through the cAMP second messenger system (Farley et al., 1992; Villa et al., 2003).

It was shown that gonadal hormone deficiency affects thyroid C cell activity. Thus, synthesis of CT and its release from rat C cells were decreased after Ovx due to lack of estrogens (Filipović et al., 2002, 2003; Sakai et al. 2000). Also, the decline in testosterone level induced by Orx altered thyroid C cell structure and reduced the synthesis and release of CT (Filipović et al., 2007; Lu et al., 2000). The same effects were noticed after Orx or the natural menopause in women (Isaia et al. 1989). On the other hand, estrogen treatment was found to have a stimulatory effect on CT secretory activity of C cells in Ovx rats (Grauer et al., 1993; Filipović et al., 2003), Orx rats (Filipović et al., 2010a) and women (Isaia et al., 1992). In addition to estrogen, chronic calcium administration after Ovx increased the release of CT from C cells without affecting CT synthesis, suggesting that estrogen plays an important role in CT synthesis (Filipović et al., 2005). On the other hand, CT administration, which may be useful for treatment of osteoporosis, negatively affected rat thyroid C cells by a negative feedback mechanism (Sekulić et al., 2005).

Among the few studies concerning the potential effects of phytoestrogens on CT production, the influence of ipriflavone, a derivative of isoflavone, on CT synthesis and secretion was

investigated. Administration of ipriflavone to intact rats had a gender-related effect on serum CT, which increased in females, but no significant change was seen in male rats (Watanabe et al., 1992). With regard to the inhibitory effect of testosterone on the synthesis of some enzymes it is possible that testosterone inhibited ipriflavone-stimulated CT synthesis (Weiner & Dias, 1990).

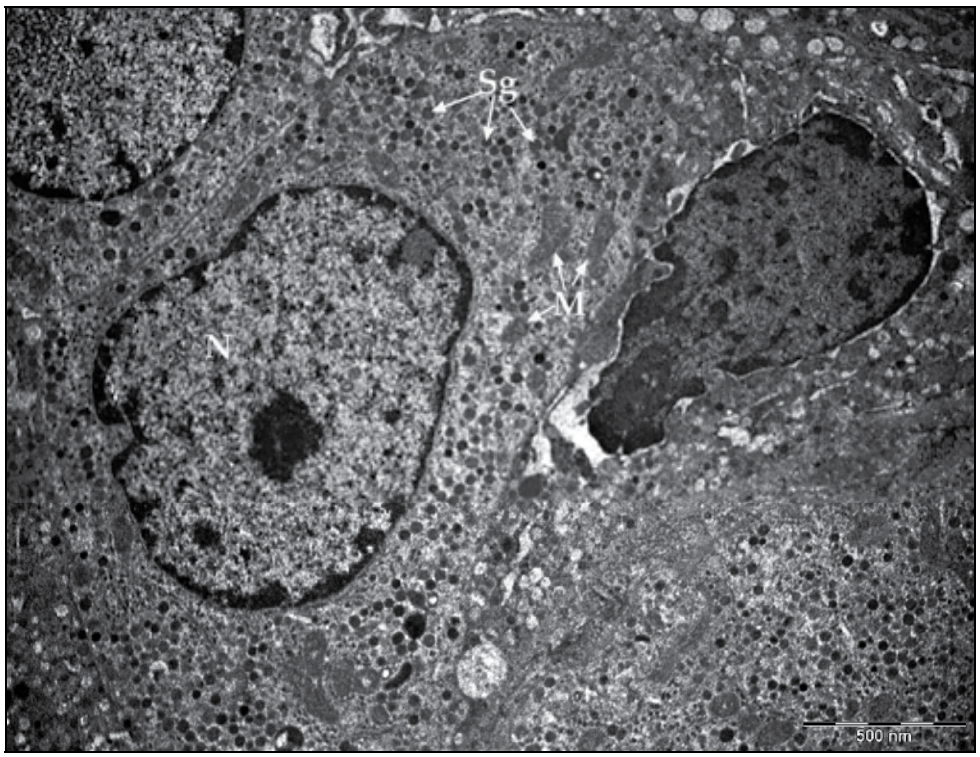

Fig. 5. Ultrastructure of a thyroid C cell; nucleus (N), mitochondria (M), secretory granules (Sg); unpublished image of Filipović et al.

Recently the first experimental data suggesting that daidzein affects thyroid C cells and stimulates CT secretory activity in Orx middle-aged rats were presented (Filipović et al., 2010). The androgen deficiency after Orx strongly affected thyroid C cell structure and reduced the synthesis and release of CT. Daidzein treatment decreased immunoreactivity for CT, significantly increased C cell volume (Fig. 6) and slightly raised serum CT concentration.

Daidzein administration also decreased bone turnover, prevented loss of cancellous bone and the plate-like structure was recovered after trabecular bone destruction caused by Orx (Fig. 7). Based on these results, the authors suggested that, besides direct action on the skeleton, daidzein may affect bone structure indirectly through enhancement of thyroid C cell activity (Filipović et al., 2010).

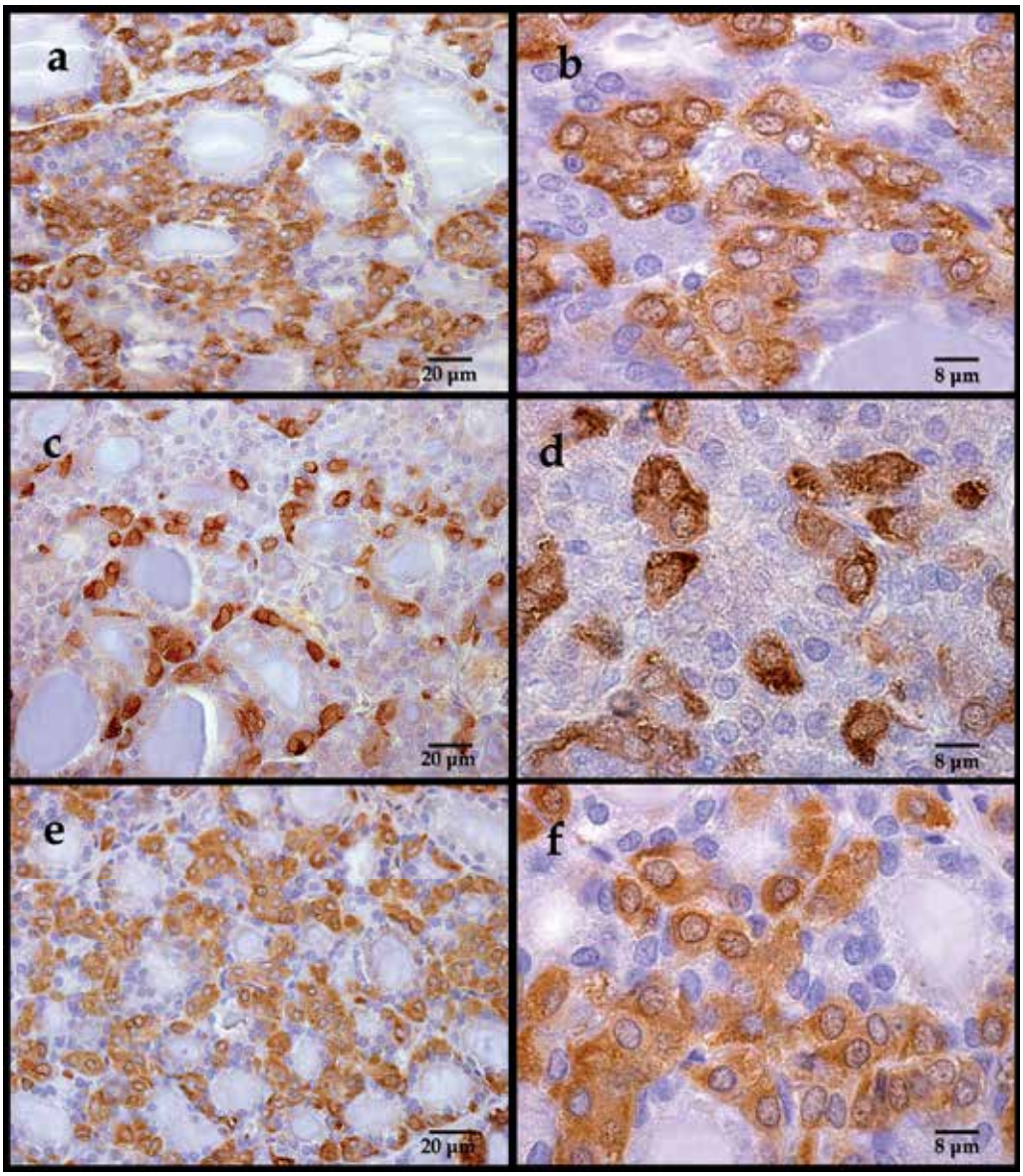

Fig. 6. Calcitonin producing thyroid C cells in control (a, b), orchidectomized (c, d) and orchidectomized rats treated with daidzein (e, f); immuno-staining for calcitonin; unpublished image of Filipović et al.

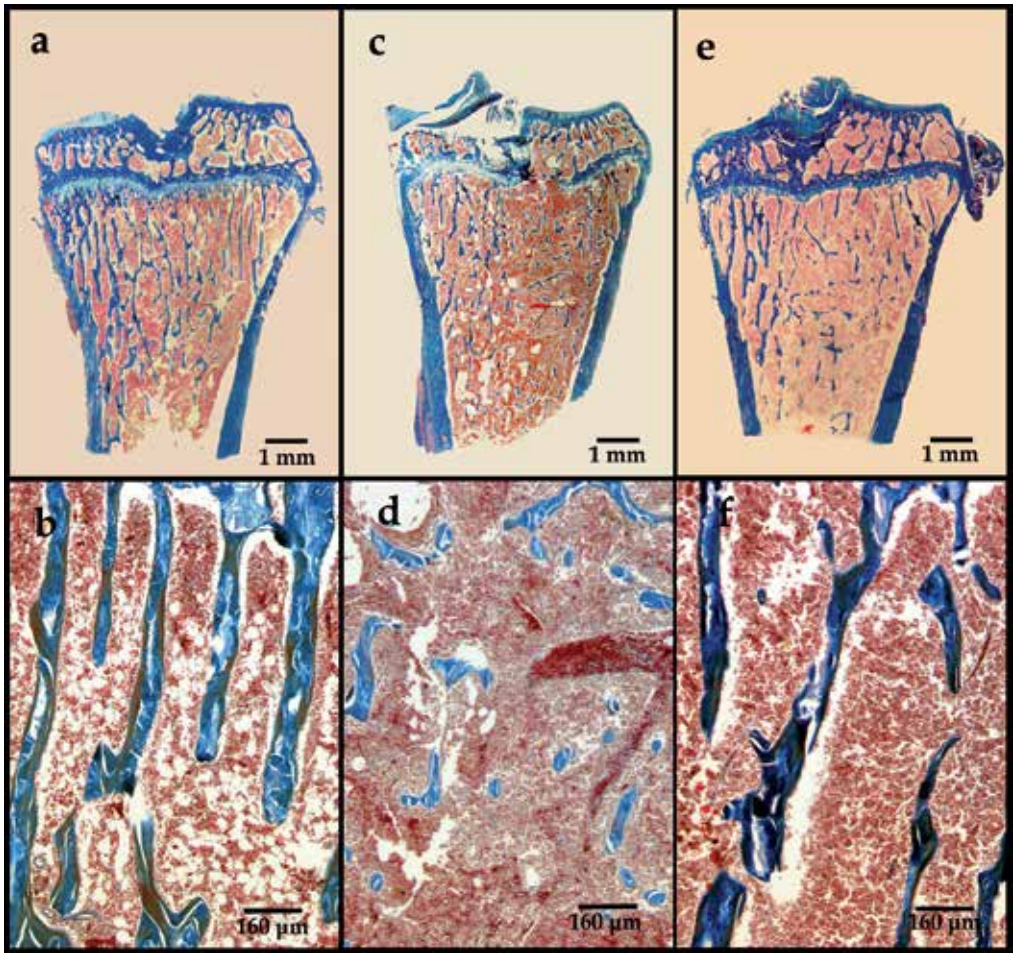

Fig. 7. Trabecular microarchitecture of the proximal tibial metaphysis in control (a, b), orchidectomized (c, d) and orchidectomized rats treated with daidzein (e, f); azan staining method; unpublished image of Filipović et al.

5.2 Effects of phytoestrogens on parathyroid hormone production

Parathyroid glands are constituted of chief, clear and oxyphilous cells. The chief cells synthesize and secrete PTH and are arranged in rather dense cords or nests around abundant capillaries. These cells are oval or polygonal in shape. The nucleus is irregularly shaped, with a few spots of chromatin located in the margin, and the nuclear membrane is infolded. The plasma membrane shows interdigitations. Mitochondria are dispersed throughout the cytoplasm. The cisternae of the rough-surfaced endoplasmic reticulum are arranged in parallel arrays or randomly distributed in the cytoplasm. The Golgi complexes are well developed. Storage granules are filled with finely particulate electron-dense material (Fig. 8).

PTH plays an important role in calcium homeostasis and has a critical role in bone turnover. It antagonizes CT produced by thyroid C cells and acts directly on bone and kidney to increase Ca influx into the blood circulation. This hormone increases the tubular re-

absorption of calcium and induces increased conversion of 25(OH)-D to 1,25(OH)2-D, which enhances intestinal calcium absorption and increases skeletal calcium mobilization.

PTH has a biphasic effect on bone, as it stimulates bone formation when given intermittently, whereas continuous infusion reduces bone mass (Kim et al., 2003). Treatment with PTH significantly increases ALP activity, which suggests that this hormone modulates SaOS-2 osteoblastic cell differentiation and has an anabolic effect on bone. However, increases in RANKL mRNA and decreased OPG mRNA expression in SaOS-2 cells due to PTH indicates induction of bone resorption (Chen & Wong, 2006).

Elevated PTH secretion contributes to the greater bone resorption in osteoporosis which is related to estrogen deficiency. Estrogen therapy prevented the increase in PTH levels associated with the menopause (Khosla et al., 1997). Similarly, phytoestrogens behave as estrogen and may prevent the bone loss caused by estrogen deficiency in female animals and women through reduction of PTH levels. It was shown that phytoestrogens from medical plants can lower serum PTH levels in aged menopausal monkeys (Trisomboon et al. 2004). Also, postmenopausal women with habitually high intakes of dietary isoflavones had significantly lower levels of serum PTH and higher BMD (Mei et al., 2001). These plant compounds bind to ERs in the kidney, gastrointestinal tract and bone and improve calcium absorption resulting in a secondary decrease in the PTH level. Moreover, phytoestrogens may directly reduce PTH secretion from the parathyroid gland (Wong et al., 2002).

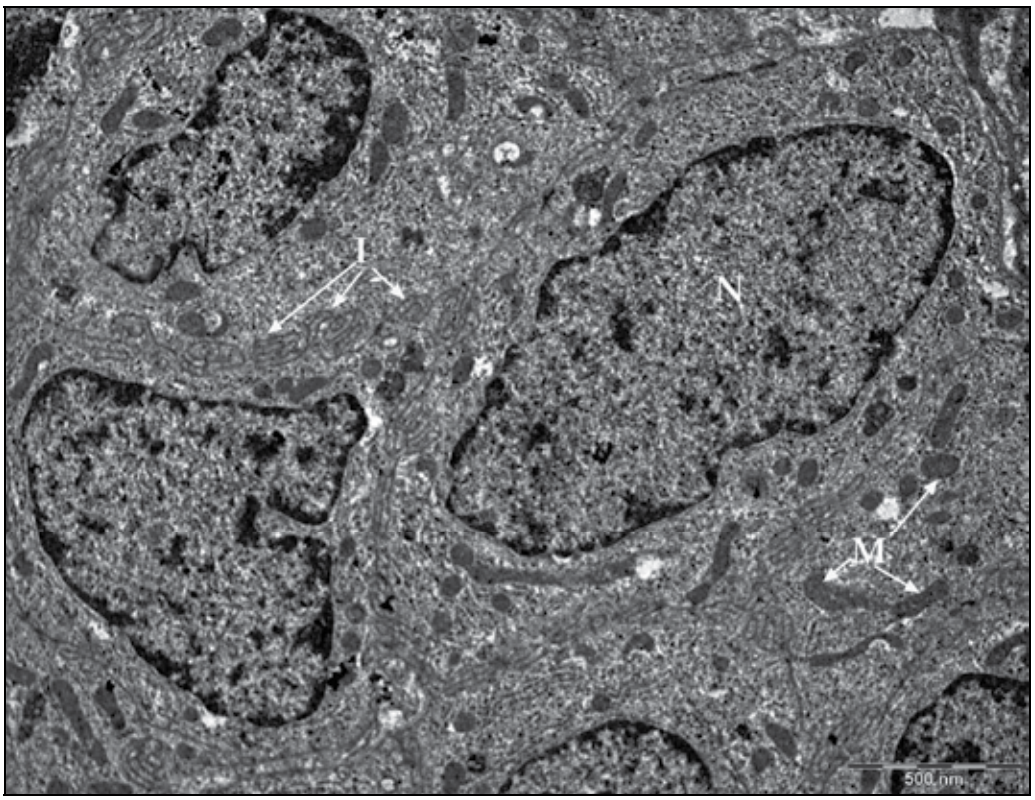

Fig. 8. Ultrastructure of parathyroid chief cells; nucleus (N), mitochondria (M), interdigitations of the plasma membrane (I); unpublished image of Pantelić et al.

Mimicking the effect of estrogen, phytoestrogens can modulate the action of PTH on bone. Thus, one study in vitro showed that pre-treatment of SaOS-2 osteoblastic cells with genistein enhanced PTH-induced ALP activity and attenuated PTH up regulation of RANKL mRNA expression and PTH down regulation of OPG mRNA expression (Chen & Wong, 2006).

5.3 Effects of phytoestrogens on thyroid glands and thyroid hormones production

Hypothalamic–pituitary–thyroid axis (HPT) plays a key role in skeletal development, attainment of peak bone mass and regulation of adult bone turnover (Gogakos et al., 2010; Roef et al., 2011). Additionally, thyroid disorders are associated with alterations in bone metabolism (Lakatos, 2003).

Soy-food, soy-based infant formula, as well as dietary supplements containing purified soybean isoflavones, genistein and daidzein, are increasingly consumed in typical "Western" diet in the recent years. Commonly cited reasons for using soy infant formula are to feed infants who are allergic to dairy products or are intolerant of lactose, galactose, or cow-milk protein (Tuohy, 2003). In elderly, reason is potential health benefit of soybean isoflavones in protection of age-related diseases, including osteoporosis (Setchell, 1998).

Structurally, soybean isoflavones genistein and daidzein are polyphenolic compounds, similar to estradiol-17β and bind with a weaker potency to both types of ERs, with higher affinity for ERβ (Kuiper et al., 1998). Despite the numerous beneficial effects of soy isoflavones, epidemiological and experimental data also exist showing an adverse effect on human health, namely on reproductive and thyroid axis. The association between high soy isoflavones intake and goitrogenesis, as well as protective effect of adequate iodine intake, was reported both in humans (Chorazy et al.1995; Van Wyk et al., 1959) and in different animal models (Ikeda et al., 2000; Kimura et al., 1976; McCarrison, 1933).

Therefore, besides the direct beneficial effect of soybean phytoestrogens on bone tissue, isoflavones may also act indirectly, through endocrine disruption and interference with HPT axis. Most researchers who examined osteoprotective potential of isoflavones did not include in their research examining of the thyroid status. We will address that aspect in this subchapter.

5.3.1 Phytoestrogens, thyroid hormones and skeletal development

Normal thyroid function in childhood is essential for development of endochondral and intramembranous bone, for normal linear growth, as well as for establishing peak bone mass. Hypothyroidism in children causes growth arrest, delayed bone maturation, and epiphyseal dysgenesis, while T_4 replacement results in rapid catch-up growth (Basset & Williams, 2003). Exposure to soybean isoflavones during development may alter thyroid hormone concentrations and disturb feedback regulation of HPT axis, and these effects can be more serious than in the adults.

Soy infant formula is fed to infants as a replacement for human milk, or as an alternative to cow milk formula. Genistein is the predominant isoflavone found in soy infant formula (58-67%), followed by daidzein (29-34%) and glycitein (5-8%) and infants fed soy infant formula have higher daily intakes of genistein and other isoflavones than other populations (Patisaul & Jefferson, 2010). The question of whether or not soy infant formula is safe has been widely debated for more than a decade, and early epidemiological studies demonstrated that infants fed adapted soy formula without iodine supply were hypothyroid (Van Wyk et al., 1959). This effect was eliminated by supplementing commercial soy infant formulas with iodine, or by

switching to cow milk (Chorazy et al., 1995). Today, soy formula is regularly supplied with iodine and a more recent study demonstrated no significant changes in the serum level of bone alkaline phosphatase, osteocalcin, intact PTH, and the urinary levels of the markers of bone metabolism in children (mean age of 37 months) fed with soy formula (Giampietro et al., 2004). However, infants with congenital hypothyroidism fed with iodine supplemented diet still need higher doses of L-thyroxine (Jabbar et al., 1997). This finding is of particular importance, keeping in mind that the consequence of congenital and juvenile acquired hypothyroidism is retardation of skeletal development and that the effects of T_4 replacement (achievement of predicted adult height) strongly depend on the duration of untreated hypothyroidism (Rivkees et al., 1988).

Soybean isoflavones may functionally disrupt the thyroid hormone (TH) system by influencing different steps such as synthesis, transport, action and metabolism of TH. Genistein and daidzein inhibit the activity of thyroid peroxidase (TPO), the key enzyme in the synthesis of thyroid hormones, both in vitro and in vivo (Chang & Doerge, 2000; Divi et al., 1997; Doerge & Chang, 2002). Besides the inhibitory effects of isoflavones on TPO, iodine deficiency is important risk factor for thyroid dysfunction and goiter development, both in humans and in rats. An adequate iodine supply is a way to prevent goitrogenic effects of soy bean isoflavones, especially in the high-risk group of patients with congenital hypothyroidism.

Besides the serum concentrations of TH, biological activity of T_3 on bone tissue is determined by the membrane transporters of TH, local expression and activity of deiodinase enzymes and receptors for TSH and TH. Polymorphisms in above mentioned genes are associated with important chronic skeletal diseases, including osteoporosis and osteoarthritis (Andersen et al., 2002, 2003; Peeters et al., 2006).

Entry of T_3 and T_4 into target cells is determined by the active uptake of free hormones by specific cell membrane transporters: monocarboxylate transporter-8 (MCT8), MCT10 and organic acid transporter protein-1c1 (OATP1c1) (van der Deure et al., 2010). MCT8 is expressed in growth plate chondrocytes, bone forming osteoblasts and bone resorbing osteoclasts at all stages of cell differentiation, and its expression is regulated by thyroid status (Capelo et al., 2009), although its functional importance is still unclear. It seems that OATP1c1 is not expressed in the mouse skeleton (Capelo et al., 2009), but there are still no data regarding expression of MCT10. Tyrosine kinase inhibitors sunitinib and imatinib inhibit MCT8 – mediated iodothyroinine transport (Schweizer et al., 2010), but there are still no data regarding possible effects of genistein, which is a potent thyrosine kinase inhibitor as well, on cellular transport of TH.

Deiodinase (Dio) enzymes determine the intracellular levels of bioactive T3 and thus cell-specific gene expression. Expression of deiodinases is tissue specific: Dio 1 enzyme is not expressed in bone, while Dio 2 plays an important role in local regulation of thyroid hormone signaling during fetal bone development. In the adult skeleton Dio 2 activity is restricted to osteoblasts (Williams et al., 2008). Dio 2 expression and activity are inhibited by high concentrations of substrate (T_4) and thus are maximal in hypothyroidism and suppressed in thyrotoxicosis. Locally regulated activity of Dio 2 in osteoblasts maintains intra-cellular T_3 concentrations constant over the euthyroid range and preserves optimal bone mineralization. Inactivating deiodinase type 3 (Dio 3) is expressed in the skeleton, although the highest levels of enzyme activity occur in growth plate chondrocytes prior to weaning (Yen, 2001). Genistein inhibit both Dio 1 and Dio 2 activity in vitro (Mori et al., 1996), but the physiological importance of this mechanism is still unclear.

Based on analyses of rare monogenic diseases and the results of animal studies, it was proposed that T_3 play a key role in bone development, while TSH is not required for normal skeletal development (Bassett et al., 2008). T_3 enters the nucleus and binds to its nuclear receptors (TR). There are three functional TRs: TRα1, TRβ1 and TRβ2, encoded by the THRA and THRB genes. These receptors act as hormone inducible transcription factors that regulate expression of T_3-responsive target genes (Yen, 2001). Both TRα1 and TRβ1 isoforms are expressed in bone and TRα1 levels are at least 10-fold greater than TRβ1. These findings support the opinion that TRα1 is the principal mediator of T_3 action in bone (Bassett & Williams, 2009; O'Shea et al., 2003).
In vitro experiments demonstrated that effects of T_3 in osteoblastic cell lines and primary osteoblast cultures depend on species, cell type, anatomic origin, differentiation phase and duration of the treatment. T_3 was reported to increase expression of osteocalcin, osteopontin, type I collagen, alkaline phosphatase, IGF-I and its regulatory binding proteins IGF1BP-2 and -4 (Milne et al., 2001; Pereira et al., 1999; Varga et al., 2004). Therefore, T_3 may exert its stimulatory effect on osteoblasts via complex pathways involving many growth factors and cytokines.

5.3.2 Phytoestrogens, thyroid hormones and osteoporosis prevention

Similar to osteoporosis, thyroid diseases are much more common in elderly women than in men and is associated with significant morbidity if left untreated (Schindler 2003; Suchartwatnachai et al., 2002). Still, this fact does not imply a causal relationship between the two diseases and many patients may independently develop both. Hypothyroidism occurs in 10% of females and 2% of males in patients older than 60 years. The prevalence of hyperthyroidism in the elderly is approximately 2% (Maugeri et al., 1996), though other authors reported that 10 to 15% of elderly patients were hyperthyroid (Kennedy & Caro, 1996). Thyrotoxicosis increase risk in developing secondary osteoporosis (Amashukeli et al., 2010; Lakatos, 2003).
Thyroid hormones play a significant role in maintaining adult bone homeostasis. Results of clinical and experimental studies are consistent and demonstrate that hypothyroid state slows down bone turnover and affect overall gain in bone mass and mineralization. By contrast, bone resorption and formation are accelerated in hyperthyroidism, while the remodeling cycle is shortened (Davies et al., 2005). Increased bone turnover and osteoporosis in thyrotoxicosis are attributed to the thyroid hormone excess and are not a consequence of deficient TSH receptor (TSHR) signaling. However, TSH may play a direct role in regulation of bone turnover, since TSH receptor was identified in osteoblasts. The experiment with ovariectomized rats, which were treated with low doses of TSH (insufficient to alter serum T_3, T_4 or TSH levels), demonstrated that TSH treatment prevented bone loss and increased bone mass (Sampath et al., 2007; Sun et al., 2008). Although the TSHR is expressed in osteoblasts, current data from in vitro studies are contradictory and suggest that TSH may enhance, inhibit or have no effect on osteoblast differentiation and function (Bassett et al., 2008).
Prevention and treatment of osteoporosis involve Ca and vitamin D supplementation, as well as different drug therapy approaches, which include bisphosphonate, salmon CT and estrogen or androgen replacement therapy for menopausal women and andropausal men, respectively. In addition, in recent years, numerous discussions on safety and benefit of synthetic steroids (both estrogens and androgens) favor the trend towards consumption of

"green" natural "phytosteroids" or "phyto-selective modulator of ERs". That is why nutritional supplements and concentrated extracts containing purified soybean phyto-SERMs genistein and daidzein are increasingly used as alternative therapy for osteoporosis and other age-related diseases in both sexes (Ramos, 2007; Setchell, 1998; Tham et al., 1998). However, all these treatments may affect thyroid function as well.

Not so many researchers have tried to link effects of supplementation or drug treatment on bone metabolism with modulation of thyroid hormone levels. Rodents are considered useful models for thyroid studies, even though significant differences between rodent and human thyroid physiology have been reported (Choksi et al., 2003; Poirier et al., 1999). Rat thyrocytes are characterized by abundant granular endoplasmic reticulum, well developed Golgi, prominent lysosomes, luminal (apical) microvilli, small mitochondria, and round nuclei with homogeneous chromatin (Fig. 9).

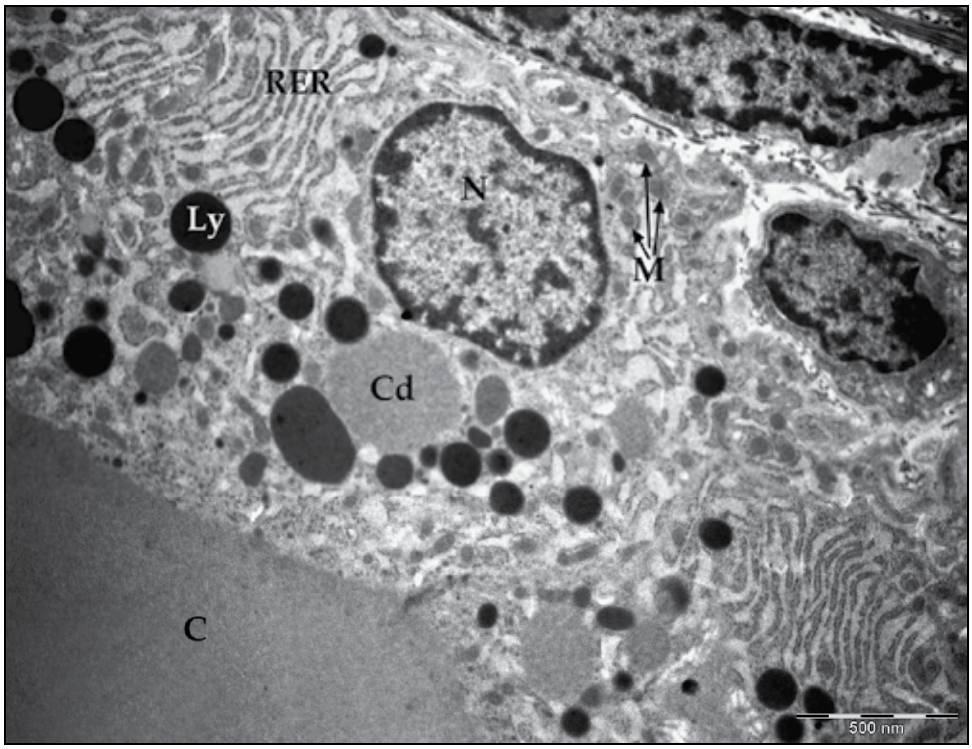

Fig. 9. Ultrastructure of thyroid follicular cell; nucleus (N), mitochondria (M), rough endoplasmatic reticulum (RER), lysosomes (Ly), colloidal droplets (Cd), colloid (C); unpublished image of Šošić-Jurjević et al.

In our laboratory we demonstrated that chronic Ca administration to middle-aged female rats significantly decreased the volume density of the thyroid follicular epithelium, epithelium's height and the index of activation rate, which are morphometric parameters of TH synthetic and secretory potential of thyrocytes (Šošić -Jurjević et al., 2002). Consistent with histomorphometric changes, reduced serum levels of total T_4 and T_3 were detected (Šošić -Jurjević et al., 2006). At the same time, we determined significant decrease of serum osteocalcin and urinary Ca, as biochemical parameters of reduced bone turnover after Ca

treatment (unpublished data). In vitro studies with FTRL-5 cells demonstrated that Ca did not affect the morphology of these cells, but when administered together with TSH, it acted directly, by reducing the thyrotropin stimulatory effect (Gaberscek et al., 1998). Isoform VI of adenilyl cyclase, the enzyme crucial for TSH-induced activation of thyroid follicular cells, was found negatively modulated by Ca in human and dog thyroids (Vanvooren et al., 2000). Doses of Ca were chosen to mimic human exposure to high doses of Ca in treatment of osteoporosis. We can speculate that slowing down of thyroid hormone synthesis may be an indirect mechanism, which lead to decreased bone turnover detected after Ca treatment under our experimental conditions.

Sex steroids, estrogen and testosterone, play an important role in bone physiology and pathology. Endogenous estrogens are regularly produced in bone via aromatase enzyme activity, and exert their effects through ER, which are also detected in male bones (Carani et al., 1997; Grumbach & Auchus, 1999; Korach, 1994). Bone cells are sensitive to both estrogens and androgens, and aromatase inhibition causes similar degree of osteoporosis in male animals as orchidectomy (Vanderschueren et al., 1998).

There is a close relationship between sex steroids and thyroid function. Epidemiological studies suggest that the use of estrogens may contribute to the pathogenesis of thyroid tumors (Ron et al., 1987). Experimental studies on rodents demonstrated numerous sex-related differences in thyroid function and, in general, adult male rodents have higher levels of TSH than females associated with lower T_4 and higher plasma levels of T_3 (Capen, 1997). The results related to treatment effects of sex steroids on different set points of thyroid function are inconsistent and depend on experimental conditions: type of experimental animal, animal's age and applied dose (Chen & Wallfish, 1978; Henderson et al., 1982; Sekulić et al., 2007). Our previous results demonstrated an inhibitory effect of pharmacologic doses of estradiol (previously used in human studies for treatment of osteoporosis) on thyroid follicular cells in ovariectomized young adult and ovarium-intact young and middle-aged rats, (Sekulić et al., 2006; Šošić -Jurjević et al., 2005, 2006a), as well as after treatments of orchidectomized 16-month-old rat males with 10 times lesser dose of estradiol dipropionate (Sekulić et al., 2010). We choose the dose of estradiol in the experiment which was previously reported to prevent bone loss in males (Fitts et al., 2001; Vanderput et al., 2001). Consistent with literature data, we also detected decreased serum osteocalcin levels, accompanied by decreased urinary Ca concentration in Orx rats treated with EDP (unpublished data). Contrary to effects of estradiol, testosterone treatment of castrated middle-aged males moderately increased serum TSH and total T_4 levels (Sekulić et al., 2010), but similarly to estradiol treatment, decreased both serum osteocalcin levels and urinary Ca concentration (unpublished data). Therefore, it seems that the direct effect of sex steroids on bone tissue is more relevant for the net result of replacement therapy on bone protection then the indirect effect, mediated through modulation of thyroid function.

Direct negative effect of isoflavones on thyroid hormone synthesis, by significant blocking of TPO activity (more than 60%), has been well described. Genistein and daidzein were demonstrated to block both TPO-catalyzed reactions: iodination of thyrosine residues of Tg, and T_4 formation by coupling reactions, but this effect was eliminated by iodine (Chang & Doerge, 2000; Divi et al., 1997; Doerge & Chang 2002). Despite significant inactivation of this enzyme, serum thyroid hormone levels were unaffected by isoflavone treatments in young adult rats of both sexes. The authors supposed that soy could cause goiter, but only in animals or humans consuming diets marginally adequate in iodine, or who were predisposed to develop goiter. Most other authors, who performed their studies on young adult animals of both sexes, also reported that soy or isoflavones alone, in the absence of

other goitrogenic stimulus, did not affect thyroid weights, histopathology and the serum levels of TSH and thyroid hormones (Chang & Doerge, 2000; Schmutzler et al., 2004). The thyroid function becomes impaired with aging in rodents, and the number of thyroid dysfunction increase in elderly population (Donda & Lemarchand-Béraud, 1989; Reymond et al., 1992). We were the first who demonstrated that therapeutic doses of both genistein and daidzein induce hypertrophy of Tg-immunopositive follicular epithelium and colloid depletion (Fig. 10), and reduce the level of serum thyroid hormones, accompanied by

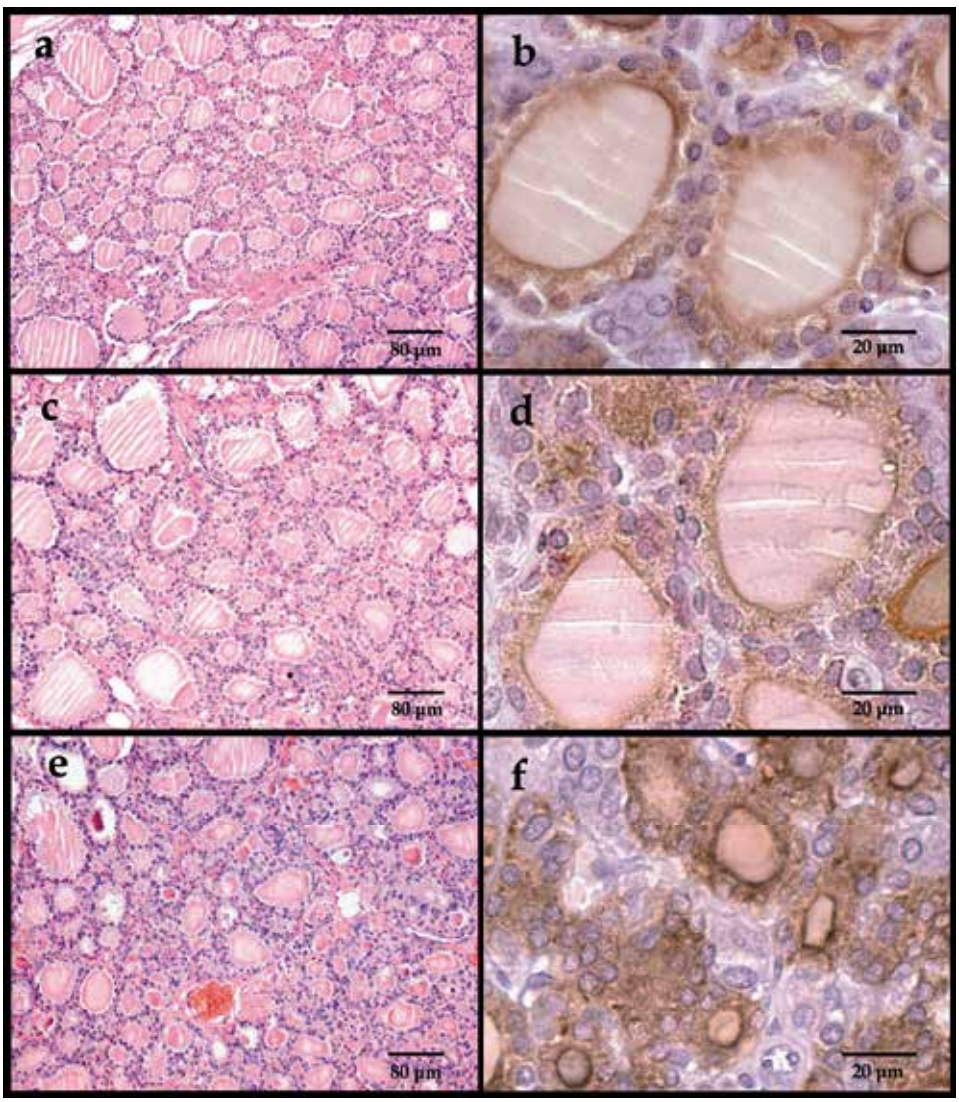

Fig. 10. Thyroid gland tissue of control (a, b), orchidectomized (c, d) and orchidectomized rats treated with daidzein (e, f); hematoxylin-eosin and immuno-staining for thyroglobulin; unpublished image of Šošić-Jurjević et al.

increased serum TSH, in orchidectomized (Orx) middle-aged male rats fed a iodine-sufficient soy-free diet (Šošić -Jurjević et al., 2010). Our research team obtained that both genistein and daidzein increased bone mass following orchidectomy of middle-aged males (Filipovic et al., 2010 and unpublished data). Therefore, decreased serum level of TH might contribute to the detected increase in trabecular bone mass, and decrease in bone turnover in aged male orchidectomized rat model.

6. Conclusion

Phytoestrogens have the potential to maintain bone health. Owing to their properties, these plant-derived non-steroidal compounds have a potential beneficial role in delaying or preventing osteoporosis. Therefore, they have attracted much attention as alternatives to HRT. As SERM, phytoestrogens may generate a bone protective effect via stimulation of osteoblastic bone formation and inhibition of osteoclastic bone resorption. Proposed molecular mechanisms are based on their ER-mediated effects. In addition to direct action, phytoestrogens can affect bone structure indirectly, by stimulating or inhibiting the synthesis of certain hormones, i.e. through increased synthesis of CT from thyroid C cells, as well as reduction of PTH and thyroid hormone levels.

7. Acknowledgment

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Nutrition for Enhancing Bone Volume in Mice

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1. Introduction

It is well known that postmenopausal osteoporosis is related to severe decreases in the serum estrogen levels. Estrogen deficiency produces an imbalance in the bone remodeling balance which is associated with a reduced bone volume and higher fracture risk. Androgen deficiency also produces osteoporosis by directly affecting bone cells. In addition, androgen was demonstrated to be present during the fetal period, and it is considered to play an essential role in the sexual differentiation of males. Therefore, both sex hormones, androgen and estrogen, are great important for bone homeostasis not only in females but also in males. This is further supported by the fact that both males and females express androgen receptor (AR), estrogen receptor-alpha (ERα) and estrogen receptor-beta (ERβ). Androgen and estrogen react with the AR and ERα and ERβ, respectively. It is thus speculated that they mutually regulate themselves during bone development and remodeling.

Up to present, various studies have examined the roles of estrogen and its receptors to explore potential methods for preventing or treating postmenopausal osteoporosis (Judd et al., 1983; Kousteni et al., 2002; Martin-Milan et al., 2010; Pietschmann et al., 2008). Recent clinical studies have reported that the prevention of hip bone fractures and vertebral deformities is highly pertinent to improving the quality of life for older people (Chang et al., 2004; O'Neill et al., 2009). To avoid these incidents, we have to try to achieve adequate peak bone mass during adolescent growth, it is also important to understand the bone growth, because this is currently an under-investigated area. In adult animal experiments, orchiectomy and ovariectomy reduced both the bone volume and density. Furthermore, the bone volume loss was induced not only in the long bones but also in the mandibular condyles (Fujita et al., 2001). In addition, we have reported that these phenomena were also shown in immature mice (Fujita et al., 2006). Therefore, the influence of sex hormones on bone remodeling has been demonstrated in the craniofacial region. In orthodontic and orthopedic treatment, it is especially, difficult to predict bone growth including craniofacial and mandibular growth during adolescence. The full nature of bone growth, in association with sex hormones remains to be fully elucidated.

The ratio of osteoporosis patient in Japan based on the diagnosed by Japanese society for bone and mineral research is reported 24% of over fifty-year-old people and it is indicated the number of patients is higher than those in USA and European countries (Orimo, 2000).

Therefore, we all recognize osteoporosis is one of serious social problems in Japan. This fact indicates that the influences of sex hormones on bone metabolism are very complicated, and the mechanism is very difficult to be understood.
Peak bone mass, in general, is acquired from childhood to adolescence, but 35% of cortical bone and 50% of trabecular bone are lost gradually thereafter after. The importance of preventive medicine thus has been gradually recognized in the field of orthopaedic surgery with a concept that peak bone mass is to be increased and saved during childhood as much as possible (Lapauw et al., 2009; Kaufman, 2009). Such an idea has been attracting a special attention in the field of clinical medicine for the prevention of osteoporosis. The importance of normal development and growth of bone until adolescence has also been reported (Karlsson et al., 2008; Wang et al., 2008). This fact suggested that if we are able to increase the peak bone mass during adolescence as possible by providing some effective nutrition for increasing bone volume, it may leads to prevent the risk and to delay the appearance of osteoporosis.
According to the prevention and treatment of guideline in osteoporosis, it was recommended to intake daily dose of calcium is higher (Rosen & Gallagher, 2011). However, the amount of calcium intake is approximately 500mg per one day in practically, and even added the consumption applied by supplement, could not reach daily objective intake.
A newly developed snack used in our experiments contains appropriate amounts of minerals (calcium and magnesium) and casein phosphopeptide (CPP) as well as soybean isoflavone which has a sex hormone-like action, which is recognized as a specified food for health, is also included. The objective of this chapter was to introduce the effects of bone remodeling including bone volume, density and strength in osteoporosis and healthy growing mice fed a special composition nutrition.

2. Nutrition

2.1 Function of nutrition

Recently, it was focused on the prevention of osteoporosis and life style related disease like a diabetic and heart disease. Under such situation, people are well considered the food function. It is well known that the function of foodstuff has mainly for nutrition and taste. Additionally, it has also the important function as a decreasing of risk in some disease. The development of foodstuff which has the specific function for living body adjustment is going forward actively in nowadays, therefore the daily food which we intake were diversify. Now it was reported that there are detected and demonstrated in some nutrition that the function of anti-thrombosis, anti-oxidation, encouragement to assimilate calcium, controlling of blood pressure and improvement of cholesterol readings as a food for special health uses and Japanese government had approved it (Yamada et al., 2008). For example, beta-carotene was known as a great nutrition because of its essential function, foodstuff factor as a provitamin, taste factor by its color in vegetable and fruit, and the role of anti-oxidation (Kim et al., 2010).

2.2 Casein phosphopeptide

CPP is also well known as a factor of encouragement to assimilate calcium. Milk and dairy products contains CPP together with functional protein like a milky basic protein and lactoferrin. Casein as a milk protein is a protein containing phosphoric acid, and is decomposed into oligopeptide of various sizes by the digestive enzyme. It became

clarified that special protein containing many phosphoric acid called CPP. Generally, calcium is the fifth abundant element by mass in the human body, where it is a common cellular ionic messenger with many functions, and serves also as a structural element in bone. And it has four stable isotopes (^{40}Ca, ^{42}Ca, ^{43}Ca and ^{44}Ca) plus two more isotopes (^{46}Ca and ^{48}Ca). 97% of naturally occurring calcium is in the form of ^{40}Ca which is a one of daughter products of ^{40}K decay, along with ^{40}Ar (Mueller & Boehm, 2011; Skulan & DePaolo, 1999). Calcium is hard to be absorbed in human body since it combines with negative ion such as a phosphoric acid in a small intestine. However, CPP prevents own insoluble and promote its efficiency of absorption in a small intestine due to a part of serine residue is phosphorylated and the ionic bond of CPP is carried out to calcium with the negative ion of phosphate group.

Moreover, the function in the promotion of absorption efficacy of CPP, it is positively applied in the form of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and is widely well-known as Recaldent. CPP from the major protein of milk have the remarkable ability to stabilize calcium, phosphate and fluoride ions as water soluble amorphous complexes that provide bioavairable calcium, phosphate and fluoride ions to the tooth. Animal and short-term human clinical trials to repair early staged of tooth decay by replacing the calcium and phosphate ions lost due to decay (Reynolds, 2009; Llena et al., 2009). Long-term controlled clinical trials also have demonstrated the efficacy of CPP-ACP is slowing the progression of dental caries and in regressing early stages of tooth decay (Andersson et al., 2007; Morgan et al., 2008). Moreover, it was reported that it is effective rather than the 500ppm sodium fluoride paste which the dentists have used conventionally for the purpose of prevention of tooth decay (Zhang et al., 2011; Altenburger et al., 2010).

On the other hand, it was reported that the calcium in food exists 50~60% in milk, approximately 20% in small fish and approximately 30% in vegetable. But, absorption efficacy of calcium in human body is not much excellent. Therefore, CPP is further developed as "food suitable for the people of calcium shortage" such as suger-free gum, tooth cream, dairy milk and another drink and Japanese tofu. However, with such professional tool, food and drink form, we speculated that it will be probably difficult to take freely and daily for the purpose of increasing the absorption efficacy of calcium.

3. Animal study

3.1 Materials and methods

Fifty male and fifty female C57BL/6J mice of twelve-week-old and thirty male and thirty female C57BL/6J mice of five-day-old (Jackson Laboratory, Bar Harbar, ME, USA) were used in this experiment and divided respectively into eight groups with ten in each sex. In the experimental groups, orchiectomy (ORX) and ovariectomy (OVX) were performed for ten mice of twelve-week-old in each gender at the beginning of experiment to create the situation of osteoporosis model. ORX and OVX were performed by means of a stereoscopic microscope (SZX9, Olympus Optical Co., Tokyo, Japan) under general anesthesia with sodium pentobarbital, whereas the control mice underwent sham operation. The detail of time schedules in adult and growing mice during a series of experiment are shown in Table 1 and 2, respectively. These animals were treated under the ethical regulations defined by the Ethics Committee, Hiroshima University Faculty of Dentistry.

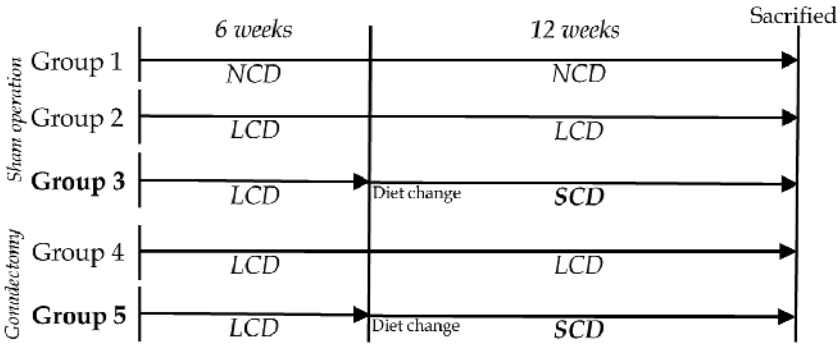

Table 1. Time schedule during experiments in adult mice

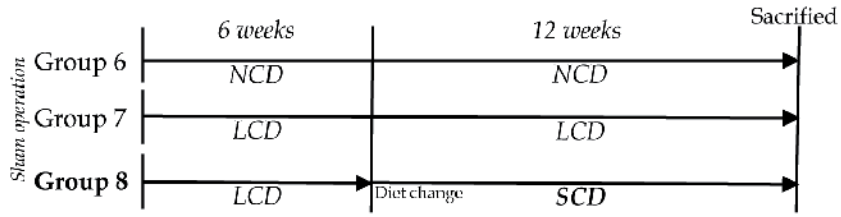

Table 2. Time schedule during experiments in growing mice

We prepared three types of powder diet *e.g.*: normal calcium diet (NCD, Ca : 0.9%, Clea Japan Co., Tokyo, Japan), low calcium diet (LCD, Ca : 0.63%, Clea Japan Co.,) and special diet (SCD, Ca : 0.9%) developed as a new bean snack. NCD with 0.9% calcium was made on basis of objective consumption of calcium intake in humans defined by the Ministry of Health, Labour and Welfare (MHLW) in Japan. LCD including 0.63% calcium was made on the basis of actual calcium consumption in Japanese. The SCD containing LCD and the newly developmental snack, was supplied as a diet including calcium content same as NCD. The newly developmental snack was composed of calcium, magnesium, CPP and black soybean. As the mainly composition, 1.3% of calcium, 0.6% of magnesium, 0.15% of CPP and 12.8% of black soybean were included in this snack. NCD was given up to six weeks after sham operation in the group 1 and 6. Other six groups were given LCD until six weeks after gonadectomy. Six weeks after surgery, group 3, 5 and 8 in each sex were given SCD including the newly developed bean snack.

Eighteen weeks after surgery, all the animals were sacrified under general anesthesia and the femur was fixed with 4% formaldehyde and prepared for histomorphometric analysis. Peripheral Quantitive Computed Tomography (XCT Research SA, Stratec Medizintechnik GmbH, Pforzheim, Germany) was used to quantify bone density and bone mineral content. Femur was measured at a point 1.4mm distal area from chondrocyte growth plate. The cortical bone area was defined as over 690mg/cm^3 threshold and the trabecular bone area was defined as under 395mg/cm^3 threshold. Moreover, bone strength of femur performed by under three point flexural test was also executed in growing mice.

We performed pairwise comparisons (Fisher) to examine the difference in measured values between the groups with a confidence level greater than 95%. All the data are presented as means±standard deviations.

3.2 In ORX and OVX mice

Figure 1 shows the photographs of femur section examined by pQCT at the end of experiment. Irrespective of the sex differences, the trabecular bone volume surrounded by the thick cortical bone was maintained at high level in the control groups, in sham operated mice fed NCD in particular (group 1). Meanwhile, trabecular bone volume was decreased moderately in the experimental group 2 with LCD as compared with control mice. Furthermore, the group 4, gonadectomized mice fed LCD exhibited an excessive decrease in bone volume as compared with the groups 1 and 2. On the other hand, the femur section in the group 5, osteoporosis model mice given SCD, presented a prominent recovery of trabecular bone volume.

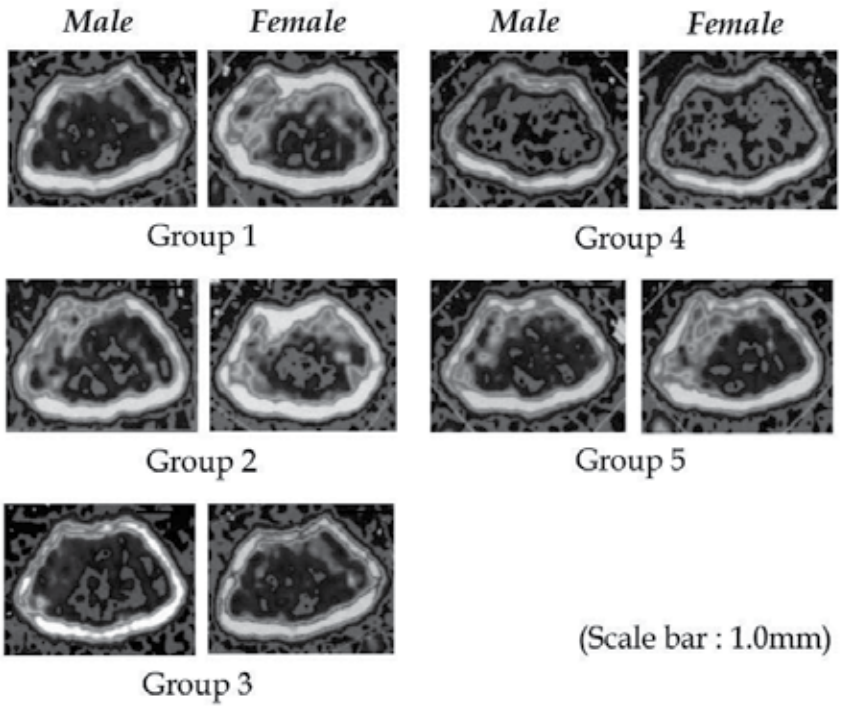

Fig. 1. Photographs of femoral section in adult mice.

As a result of pQCT analysis, similar phenomena were observed as mentioned above for the femur structures. The trabecular bone density of gonadectomized mice fed SCD was significantly increased as compared with sham opereated mice given LCD in both genders (Fig. 2). Moreover, the bone density was also significantly increased only in the female mice of group 5 as compared with sham operated mice fed NCD.

For the cortical bone density, different findings from above mentioned results were obtained. Improvement effect was especially revealed in the gonadectomized mice given SCD (group 5) (Fig. 3). Additionally, it was also shown in the sham operated mice fed SCD (group 3) compared with the sham operated mice fed LCD (group 2). These tendencies were observed in both sexes.

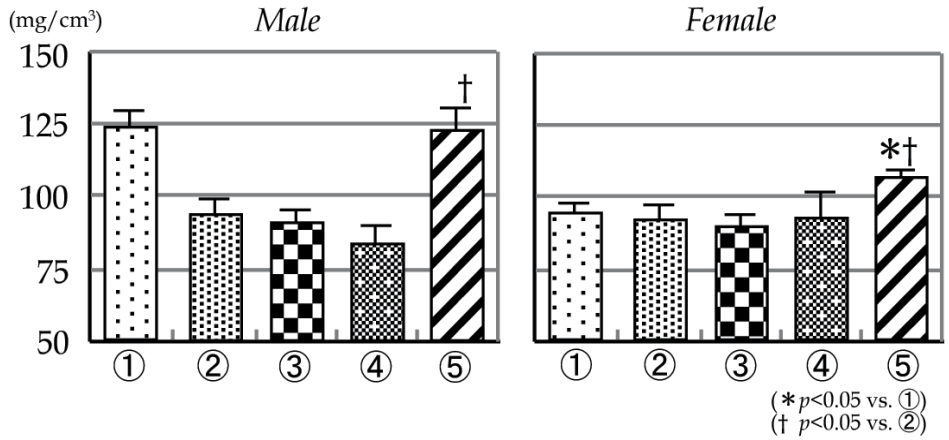

Fig. 2. Trabecular bone density in adult mice

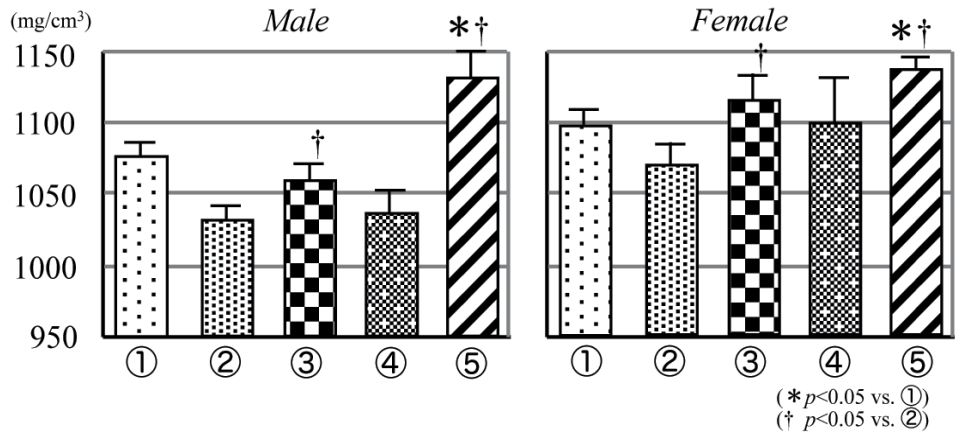

Fig. 3. Cortical bone density in adult mice

Subsequently, the total bone density was apparently increased as compared with other sham operated mice. The reason may be due to SCD supplied to the gonadectomized mice, especially in the female mice (Fig. 4).

For bone mineral content, the groups 3 and 5 mice given SCD exhibited a significant increase as compared with the groups 1 and 2, especially in female mice. It is demonstrated from these findings that the newly development snack is very effective for the improvement of reduced bone quality and controlling of osteoporosis (Fig. 5).

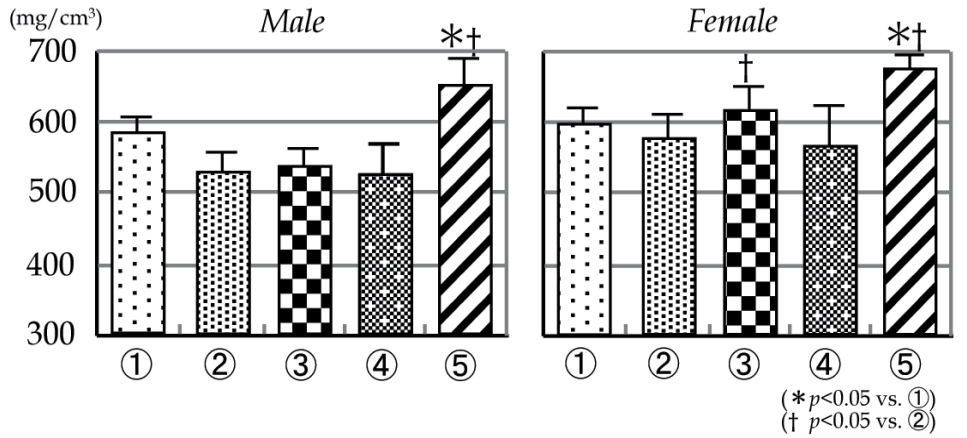

Fig. 4. Total bone density in adult mice

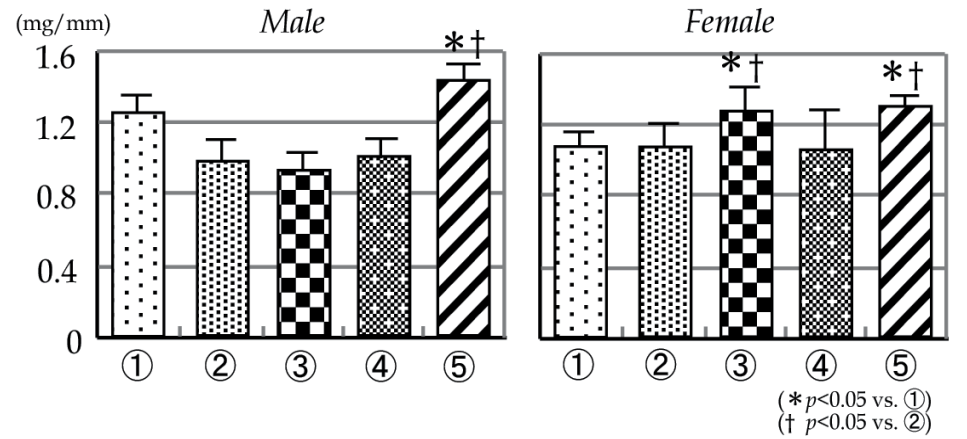

Fig. 5. Bone mineral density in adult mice

3.3 In young mice

The results of trabecular bone density were shown in Figure 6. The mice fed NCD revealed around 40 to 50 mg/cm3 in density, but by the cause of changing food to LCD, it lead to decrease in both sexes. Under such situation, in case of group 8, mice fed SCD from after six weeks to the end of experiment, trabecular bone density showed significantly increased

compared with mice fed LCD, therefore, there is no significant differences between control mice of group 6 and mice fed food which is changing to SCD after feeding LCD of group 8. In the results of cortical bone density, similar phenomena were observed with trabecular bone density, the cortical bone density of mice fed LCD revealed significantly decreased compared with mice given NCD (Fig. 7). However, by the feed changing to SCD, cortical bone density wasn't getting better as contrasted with the results of trabecular bone density and the results of cortical bone density in adult mice. These tendencies were observed in both sexes. As a result, only in female mice, total bone density also showed the sufficient effect of improvement (Fig. 8). There is no significant difference between group 6 and 8 in male mice.

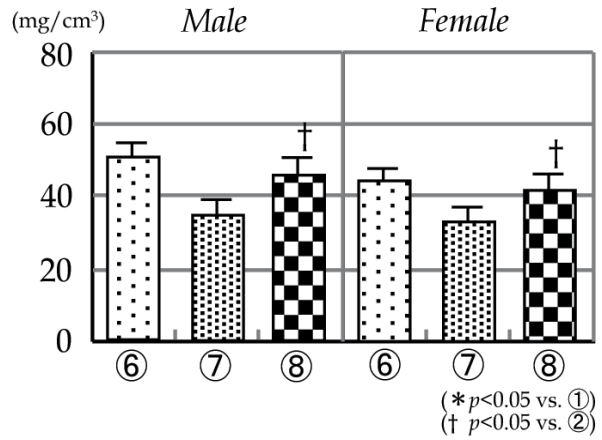

Fig. 6. Trabecular bone density in growing mice

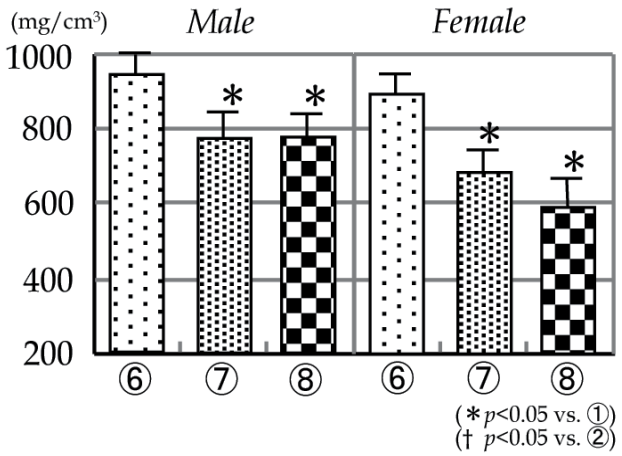

Fig. 7. Cortical bone density in growing mice

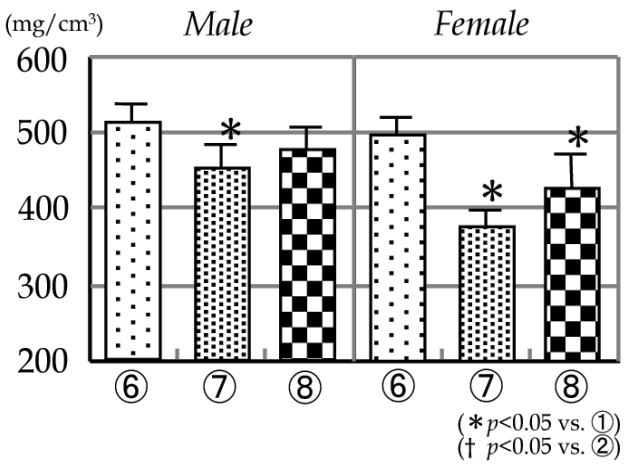

Fig. 8. Total bone density in growing mice

In the results of bone mineral content, Male mice after fed SCD showed no significant differences as compared with the control mice (Fig. 9). On the other hand, female mice of group 7 and 8 showed significantly reduced bone mineral content as compared with mice of group 6.

According to the results of total bone density and bone mineral content, the results of three point flexural test also showed same tendency in male mice (Fig. 10). Meanwhile, although the results of group 8 showed significant lower than the one of group 6 in total bone density and bone mineral content, group 7 and 8 demonstrated same level of group 6.

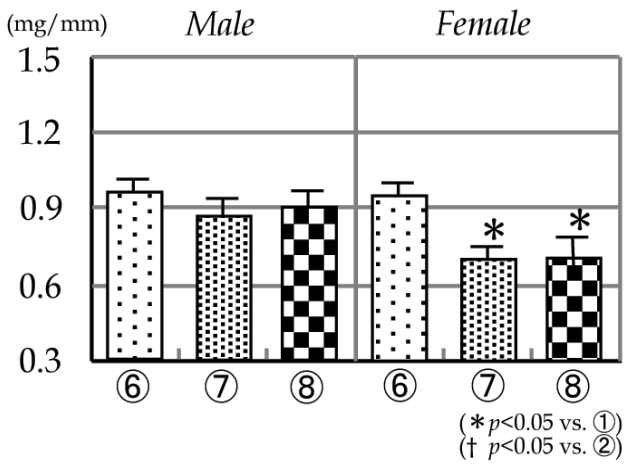

Fig. 9. Bone mineral content in growing mice

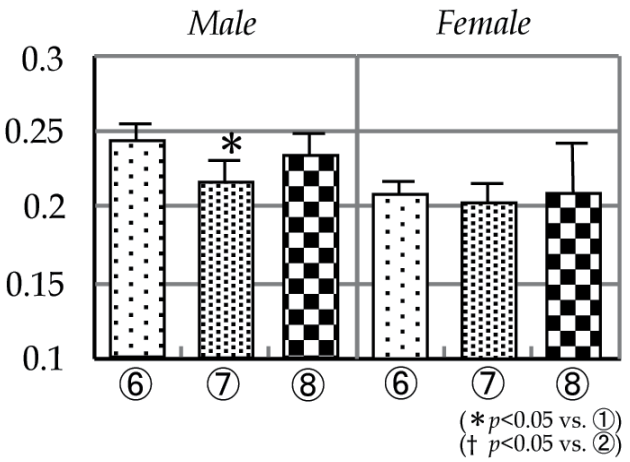

Fig. 10. Three point flexural test in growing mice

4. Discussion

Osteoporosis is generally thought of as a disease that affects females, because the prevalence of osteoporosis and the rate of fracture are much higher in postomenomausal females than in older males. However, the absolute number of male patients affected by osteoporosis and fractures has been reported to be large. Older Asian males with low serum estradiol levels also display elevated bone loss and increased risk of fractures similar to the findings in Caucasians (Woo et al., 2011; Moayyeri et al., 2009). The main concern of treatment in osteoporosis research is to prevent bone volume loss by decreasing the progression of bone resorption. Nevertheless, the current knowledge is not sufficient to identify the precise causes of osteoporosis and all of the subjects at risk. As a result, recent studies have indicated that weight-bearing activity and possibly calcium supplements are beneficial if they are begun during childhood, preferably before puberty. The achievement of optimal peak bone mass is important to prevent the risk of bone fracture due to osteoporosis in the future.

The sex hormones are known to be important for the regulation of reproductive functions. They induce sexual differentiation before birth, and sexual maturation during puberty in both genders. They also exert influences on the nervous and cardiovascular systems and are important in the development of the skeletal structure. There are two types of sex hormones, androgens and estrogens. These hormones are secreted mainly from the testis and ovaries. Testosterone can activate the androgen receptor either directly or indirectly after conversion to DHT by 5α-reductase. Moreover, it is well known that testosterone is also converted into 17β-estradiol by P450 aromatase, which activates the estrogen receptors (alpha and beta). Based on this facts, Ovariectmized and Orchiectomized animals provide an excellent model to study osteoporosis due to estrogen and androgen deficiency in both genders. Both OVX and ORX mice exhibit marked bone loss with increased bone resorption. It was also reported that a loss in bone mineral density in ORX mice was evident between 1 to 4 months post-surgery, and histomorphometric evaluations revealed that this occurred more rapidly and with greater sensitivity in a rat model.

This snack we had developed was composed of calcium, magnesium, CPP and black soybean. We have examined into body weight and height of all mice during experimental period. However, all mice of supplied food in these measurements were no significant difference compared with the control groups in both sexes (data were not shown). In addition, the amount of all nutrition including supplied food was restrained according the instructions of MHLW in Japan. Therefore, we assumed that supplied special diet food has no occurrence of side effect following snack administration.

It is well known that calcium intake decreases the risk of bone fracture. It was also reported that the calcium supplementation suppress bone formation when magnesium is deficient (Mora & Gilsanz, 2003). Based on these reports, we designed to mix calcium and magnesium at an appropriate proportion of 2 to 1. These contents in this snack were compounded to fulfill ninety percentage of the daily-required nutrition defined by MHLW in Japan.

It was also reported that addition of CPP will have a beneficial effect on the absorption of calcium (Dontas & Yiannakopoulos, 2007). In Japan, CPP was accredited as a food for qualified health uses by MHLW. Accordingly, we add a certain amount of CPP to absorb mineral content easily.

The black soybean contains isoflavone which is widely accepted to have a weak estrogen activity, and to be able to bind estrogen receptor. Actually, Brandi and Miyauchi et al., reported that isoflavone had either effect for the suppression of bone resorption or enhancement of bone formation, affecting directly both osteoclasts and osteoblasts (Brandi, 2003; Miyauchi et al., 1996). In addition, isoflavone was revealed to affect bone metabolism similarly to the sex hormone-like effect in male (Chavarro et al., 2008). Recently, it was also reported that isoflavones have revealed inhibition of bone loss in castrated male mice and growing male mice respectively (Fujioka et al., 2007; Ishimi et al., 2002; Khalil et al., 2005). Under such background, a new snack was developed by use of black soybean. The amount of isoflavone in this snack was determined with a special reference to the amount of safety surplus nutrition per day defined by MHLW.

Decrease in the trabecular and cortical bone volumes after gonadectomy has already been demonstrated by our previously reports (Fujita et al., 2001, 2004). We also reported that bone growth was significantly suppressed in the gonadectomized mice immediately after birth (Fujita et al., 2006). Moreover, we clarified that decrease in bone volume was occurred four weeks after gonadectomy. According to these results, we examined bone density of the femur six weeks after ovariectomy and orchiectomy.

The density of trabecular and cortical bones in the gonadectomized mice given LCD was significantly lower than in the sham operated mice given NCD and LCD, respectively. It was revealed that the deficiency of calcium intake caused decrease in bone density, under sex hormone disturbances in particular. Therefore, it is speculated that the bone density is below the optimal level in Japanese and fracture risk may become higher for aged people. These findings also support that the incidence of primary osteoporosis is higher in Japan than in American and European countries.

The bone metabolism is classified into two types, high turnover type to accelerate both bone formation and resorption and low turnover type caused by degradation of bone formation. In this study, twelve-week-old mice with sham operation fed LCD are regarded as young growing humans with low turnover type, whereas gonadectomy mice to simulate sex hormone disturbance in the experimental groups are assumed to be under the condition of

postmenopausal osteoporosis as a high turnover type. Irrespective of the turnover types, however, the bone density in the group 5 given appropriate amount of calcium by supplying SCD exhibited a remarkable increase. It is thus suggested that sufficient calcium quantity through nutrition of newly developed bean snack enhanced bone formation irrespective of age.

It is reported that postmenopausal women with daily calcium intake of less than 400mg experience significant bone loss and that calcium intake of 800mg per day is effective for improving postmenopausal bone loss (Dawson-Hughes et al., 1990; Reid et al., 1993). On the other hand, it is well known that improvement effect against bone volume loss by calcium intake is available only at the initial stage of treatment. In addition, Riggs et al., clarified that the effect on bone loss is weaker than those reported for estrogen and bisphosphonates therapy, indicating that calcium supplements alone can't substitute treatment for osteoporosis (Riggs et al., 1998). In this study, longitudinal effect of newly developmental snack intake was not examined. However, It is hopefully anticipated that new bean snack could contributed to the enrichment of QOL as a nutrition function food, because of it was contain of several ingredients to promote assimilation efficiency of calcium.

5. Conclusions

The new snack we developed included proper amount of calcium, magnesium, CPP and black soybean. When this product was given to the osteoporosis model mice, bone density of the femur was significantly increased. From these results, it is suggested that this product supplement promote bone formation irrespective of gender and age. We demonstrated that newly developmental snack supplements may be a useful preventive measure for the people whose bone mineral density values are less than the ideal condition.

6. Acknowledgement

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Osteoporosis and Bone Regeneration

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1. Introduction

Majority of skeletal conditions generate or heal normally due to inherent capacity. However, compromised conditions such as induced by congenital or acquired diseases may sometimes lead to uncompleted development or regenerate. The quality and volume of bone is an important factor to be considered in orthopaedic and dental fields. We occasionally encounter difficult clinical cases because of insufficient bone. Bone is a tissue that is being constantly remodelled, and bone mass at any given time depends on the balance between the rate of osteoblastic bone formation and osteoclastic bone resorption. These cellular functions are controlled by various systemic and local factors.

Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture (1993). The term osteoporosis was first introduced in France and Germany during the last century, meaning "porous bone" and initially implied a histological diagnosis, but was later refined for bone which was normally mineralized but reduced in quantity. Estrogen deficiency in postmenopausal women elicits bone loss in the vertebrae and long bones resulting in bone fractures, and this condition is called postmenopausal osteoporosis (T.J. Wronski et al., 1985, 1988, 1989a).

To the anatomical site features, a bone defect repair rate is mainly dependent on the bone wound size (J.P. Schmitz & J.O. Hollinger, 1986). Theoretically, an experimental osseous injury performed to study repair mechanisms should be wide enough to preclude a spontaneous healing. In order to do this, the non-regeneration threshold of bone tissue was investigated in the studied models, inducing a so-called critical-sized defect (CSD). The CSD may be defined as "the smallest size intraosseous wound in a particular bone and species of animal that will not heal spontaneously during the lifetime of the animal" (J.P. Schmitz & J.O. Hollinger, 1986; J.O. Hollinger & J.C. Kleinschmidt, 1990). The CSD may be therefore considered the prototype of discontinuity defects, as a condition of failed osteogenesis for overcoming the threshold of physiologic repair processes.

Although autologous or allogous bone transplant is partially effective, a simple and effective method for bone augmentation and regeneration is clinically desired in the orthopedic and dental fields. In the 1960s, Urist proposed the existence of bone-inducing molecules, which he termed bone morphogenetic proteins (BMP) (M.R. Urist, 1965). In 1988, the cDNA of BMPs was characterized, and human recombinant BMP2 (rhBMP2) (H.D. Zegzula et al., 1997; D.L. Wheeler et al., 1998; R.D. Welch et al., 1998; J.R. Lieberman et al., 1999; J.L. Dragoo et al., 2003) and BMP7 (X. Chen et al., 2002; F.C. den Boer et al., 2003; M.M. Abu-Serriah et al., 2004) are currently available. However, large amounts of rhBMP2 or 7 are required for

use in clinical treatment. Although treatment with rhBMPs is effective, the extremely high cost of their clinical application is a barrier to their use. Development of an efficient carrier for BMPs or characterizing inhibitors of BMPs and blocking them may solve this problem in the future. Furthermore, fibroblast growth factor (FGF) family members also participate in regulating osteogenesis during fracture repair. FGF2 has been shown to be widely secreted around the fracture site in the processes of wound healing and bone regeneration; however, FGF4, which plays important roles in bone development during embryogenesis, has not yet been detected in such cases in postnatal ages.

In this chapter, several histological events concerning bone are demonstrated: first, an osteoporotic effect on mandibular bone is introduced; second, calvarial healing in the bone defect was demonstrated by GBR; and subsequently, it is shown that systemic and local deliveries of FGF4 and locally carried BMP2 can contribute to osteognesis in animal experiments.

2. Experimental cases and discussion

2.1 Osteoporosis of mandible

2.1.1 Background

Bone is constantly remodelled, and bone mass at any given time is controlled by bone formation and bone resorption. These cellular events and functions are intimately associated with various systemic and local factors. Estrogen deficiency in postmenopausal women elicits bone loss in the vertebrae and long bones resulting in bone fractures, and this condition is called postmenopausal osteoporosis (T.J. Wronski et al., 1985, 1988, 1989a). Compared with the vertebrae and long bones, considerably less information is available on bone loss of the mandibles under estrogen deficient conditions. The bone mineral content of edentulous mandibles decreases with aging especially post menopause, but this is not evident in elderly men (E. Klemetti et al., 1993a, 1993b; C.W. Ulm et al., 1994; H. May et al., 1995). The ovariectomized (OVX) rat provides an experimental model of postmenopausal osteoporosis. It has been introduced that the bone mineral content and mechanical properties of the mandibles of OVX rats are similar to those of sham-operated rats; however, maxillary molar extraction causes bone loss from the mandibles of OVX rats (R.P. Elovic et al., 1994, 1995; E. Klemetti & P. Vainio, 1994). These previous studies indicate that estrogen deficiency somehow affects the mandible in both humans and experimental animals. To investigate precise region of the affected mandible, rats were ovariectomized and then subjected to longitudinal scanning for bone mineral density (BMD) measurement.

2.1.2 Materials and methods

Adult female Sprague–Dawley rats were bilaterally OVX- or sham-operated under Nembutal anaesthesia. The mandibles and femurs were dissected out after 3 months immersed in 70% ethanol. The uteri were also retrieved and weighed to check the effects of ovariectomy. Soft X-ray images of the mandibles and femurs were taken with a soft X-ray radiographic apparatus (SPOM50; Sofron, Tokyo, Japan). Total femoral BMD was initially measured with a DEXA (DCS-600R; Aloka, Tokyo, Japan), and then calculated. Total mandible BMD was measured using DEXA, after which the BMD of the incisal edge and condylar region of the mandible was measured. The trabecular and cortical BMD of the femurs were measured at cross sections 2 mm and 17 mm from the growth plate, respectively, perpendicular to the long axis with peripheral quantitative computed tomography (pQCT) (XCT 960 A; Stratec, Pforzheim, Germany) (Fig. 1). The positions are

trabecular and cortically rich regions, respectively. Also, the mandibles were scanned from the medial plane to the distal plane of the molar region with pQCT at interval of 0.5 mm as indicated in Fig. 1, following which the trabecular BMD and cortical BMD of the mandibular sections, excluding the incisor and molar, were measured using computer software.

2.1.3 Results

In OVX rats, radio opacity of the femur attenuated after OVX was observed by soft X-ray radiography, and both uterus weight (data not shown) and femoral total BMD in DEXA analysis (Fig. 3) decreased by 80.8 and 13.7%, respectively. Although the total bone mineral density and the incisor mineral density of the mandible of the ovariectomized rats were similar to those of the sham-operated rats, the bone mineral density of the condylar region in the ovariectomized rats had markedly decreased by 14%. In pQCT analysis, decrease of 30% at the 17-mm section in trabecular BMD of the femur was prominent in OVX rats whereas ovariectomy did not affect BMD of the cortical bone of the femur. The molar region of the mandible excluding the molar showed decrease of maximal 13% in trabecular bone mineral density at the eighth and its adjoining slices; on the hand, cortical BMD was not affected in any of the slices (data not shown). This study revealed regional differences in bone mineral density decrease in the mandible in ovariectomized rats.

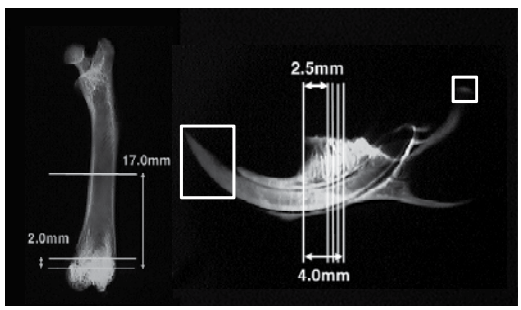


Fig. 1. Areas and positions for BMD measurement (S. Kuroda et al., 2003).

External incisal ridge and mandibular condyle in the areas surrounded with rectangles (4x6 mm) and square (2x2 mm) were scanned by DEXA, respectively. The sections including molars at interval of 0.5 mm were scanned by pQCT. Trabecular and cortically rich regions of the femur were scanned at 2 mm and 17 mm from the growth plate, respectively.

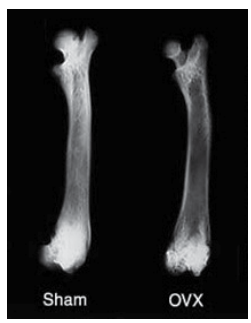


Fig. 2. Soft X-ray Radiography of the femurs. Radio opacity decreased after OVX (S. Kuroda et al., 2003).

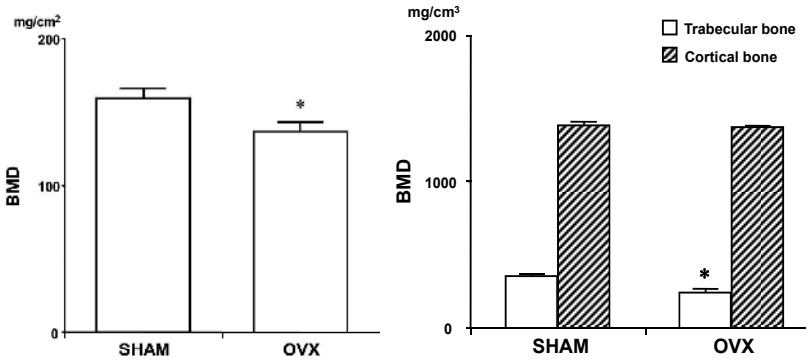


Fig. 3. (A) Total bone mineral density of femur by DEXA. (B) Trabecular and cortical bone mineral densities of femur at the sections shown in Fig. 1. Values are presented as Mean ± SD, n = 6. *p < 0.05 was regarded as statistically significant. (S. Kuroda et al., 2003)

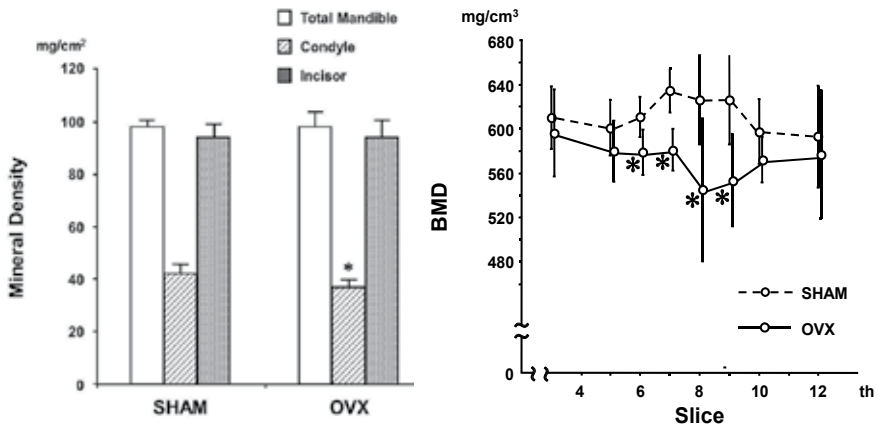


Fig. 4. (A) The total mandible, the external part of incisor and the condylar region of mandible by DEXA. (B) Trabecular bone mineral densities of mandible at the sections shown in Fig. 1. Values are presented as Mean ± SD, n = 6. *p < 0.05 was regarded as statistically significant. (S. Kuroda et al., 2003)

2.2 Guided bone regeneration with a collagen membrane

2.2.1 Background

Guided Bone Regeneration (GBR) technique is clinically used to acquire the sufficient bone volume, which has been developed by Nyman and Dahlin (C. Dahlin et al., 1988, 1989, 1990). The concept of this technique is that the application of a membrane creates a secluded space to facilitate proliferation of angiogenic and osteogenic cells from the basal bone into the defect without interference by fibroblasts. The membranes for GBR are mainly divided into three types: expanded polytetrafluoroethylene (ePTFE), synthetic biodegradable polyeters and collagen. Collagen is a material of resorbable membranes, which has several advantages such as hemostatic function, allowance of an early wound stabilization, chemotactic properties to attract fibroblasts and facilitating nutrient transfer. Therefore, collagen membranes are currently the membrane of choice for most GBR procedures.

2.2.2 Materials and methods

To investigate the efficacy of the collagen membrane for enhancement of bone regeneration in rat parietal bone defects, two symmetrical full thickness bone defects (5 mm diameter) were created at the calvarial bone of adult male Wistar rats. The defects were covered with a collagen membrane (Koken Tissue Guide, Koken, Japan) for GBR for 1 to 12 weeks. And then the specimens of the bone defect along with surrounding bone and soft tissues were collected and denuded of the skin. The samples were subjected to X-ray imaging using a μCT scanner (InspeXio; Shimadzu Science East Corporation, Tokyo, Japan) with a voxel size of 70 μm/pixel. Tri/3D-Bon software (RATOC System Engineering Co. Ltd, Tokyo, Japan) to make a 3D reconstruction from the resulting set of scans, and were also analysed by DEXA to measure the bone morphology in the defect area.

After radiographical analyses, the samples were fixed in 10% neutralized formalin for 1 week, followed by decalcification in 10% EDTA for 4 weeks. After decalcification, an incision was made precisely through the midpoint of the bone defects to ensure that the microtome sections were made in the ROI and dehydrated in ascending grades of ethanol. The samples were consequently embedded in paraffin to allow for the preparation, staining with hematoxylin–eosin, and observation of 5-μm-thick coronal sections under an optical microscope (BZ-8000; Keyence, Osaka).

2.2.3 Results

The swelling and scabbing at the incised area attenuated by 2 weeks. Alteration of the skeletal defects was visualized in the μCT images over the period (Fig. 5). The opacity of newly formed bone was not found to reach the level of the surrounding host bone in the defects of the collagen group, showing incomplete healing at 4 weeks and bone recovery became abundant at 8 weeks after surgery (Fig. 5); and on the other hand, although new bone apposition was observed partially in the control defects, they acquired newly generated bone only along the defect rim with the similar opacity to that of the collagen covered defect and did not heal completely afterward.

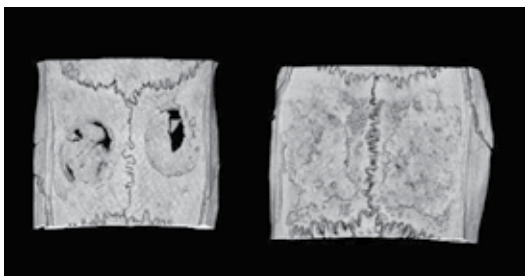


Fig. 5. μCT images of the parietal bone covered with the collagen membrane at 4 weeks (right) and without membrane at 8 weeks (left) after surgery.

Observation of the bone development process evenly and gradually replaced the collagen membrane. As shown in Fig. 6, bone regeneration as well as membrane absorption was evident in the collagen group, but the thickness of the new bone was significantly higher for the control group over the study period. Notably, osteogenesis was observed to occur primarily inside the collagen membrane.

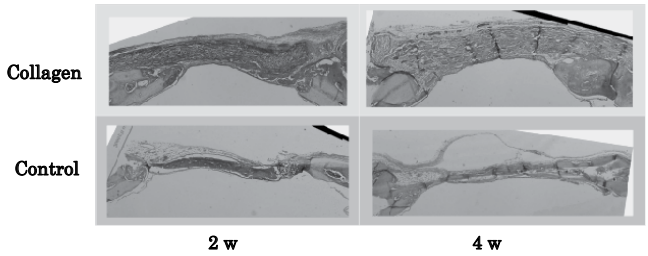


Fig. 6. Photomicrographs of the calvarial defects at 2 and 4 weeks (Hematoxylin and eosin, original magnification ×4).

The bone regenerate with the membrane was significantly prominent with BMC of newly formed bone in the defect at 8 and 12 weeks after surgery. Furthermore, the BMD of newly formed bone was significant at 12 weeks. These results indicate that covering the bone defects with collagen membranes has an ability to deliver the suitable space for bone regeneration and make the regenerated bone better quality (Fig. 7).

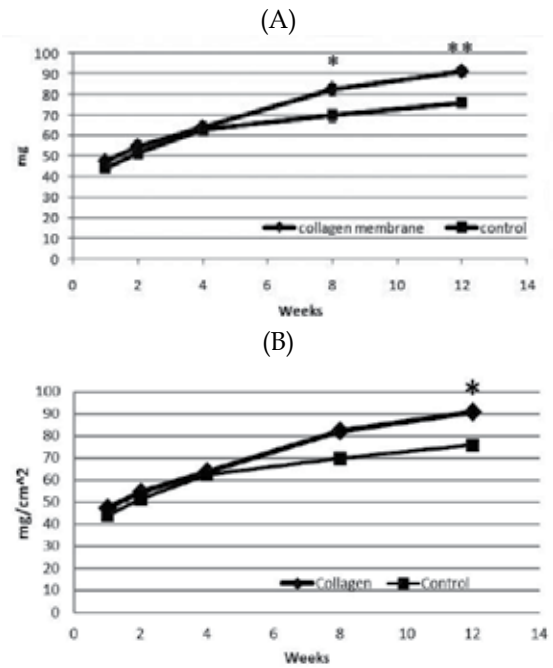


Fig. 7. (A) BMC of the defects by DEXA. (B) BMD of the defects by DEXA. *Statistically different from the uncovered control, $p < 0.05$.

2.3 Osteoconductive/osteoinductive proteins stimulate bone

2.3.1 Background

Gene expressions and productions of cytokines and growth factors in local regions that were traumatically or surgically injured are very crucial for tissue regeneration and engineering. However, cascades of the mechanisms and interactions of their roles have not been completely represented. Therefore, progress of such studies may lead to therapeutic aid.

Several FGF family members exert anabolic effects in bone when either systemically administered or locally applied (P. Aspenberg & L.S. Lohmander, 1989; H. Kawaguchi et al., 1994; T. Nakamura et al., 1995, 1998). FGF4 consists of 206 amino acid residues (M. Taira et al., 1987, T. Yoshida et al., 1987) and it has been reported that the FGF family plays a major role in the stimulation of cellular proliferation (M. Seno et al., 1990). In this study, the effects of rhFGF4s were clarified in mice after its systemic injection and in rat femurs after local administration.

In 1988, the cDNA of BMPs was characterized (J.M. Wozney et al., 1988). Gene therapy using genes of osteogenic proteins, such as BMPs, has been a focus of considerable attention (J. Fang et al., 1996; J. Bonadio et al., 1999, 2000; J.R. Lieberman et al., 1999; R.T. Franceschi et al., 2000; N. Abe et al., 2002; Y. Chen et al., 2002; J.L. Dragoo et al., 2003; C.H. Rundle et al., 2003; H. Tsuda et al., 2003; A.L. Bertone et al., 2004; I. Ono et al., 2004). When osteogenic genes are transferred to local cells, protein secretion begins and the stimulation of osteogenesis by the protein continues for a longer time than that seen in protein therapy. Experimental studies on osteogenic gene transfer have been emerging, and there are several gene transfer techniques used to stimulate bone regeneration: *ex vivo* (J.R. Lieberman et al., 1999; R.T. Franceschi et al., 2000; N. Abe et al., 2002; Y. Chen et al., 2002; J.L. Dragoo et al., 2003; C.H. Rundle et al., 2003; H. Tsuda et al., 2003; A.L. Bertone et al., 2004) and *in vivo* (J. Fang et al., 1996; J. Bonadio et al., 1999; J. Bonadio, 2000; K. Honma et al., 2001; H. Uusitalo et al., 2001; A. Sano et al., 2003; I. Ono et al., 2004) gene transfers and gene transfers with viral vector (J.R. Lieberman et al., 1999; R.T. Franceschi et al., 2000; H. Uusitalo et al., 2001; N. Abe et al., 2002; Y. Chen et al., 2002; J.L. Dragoo et al., 2003; C.H. Rundle et al., 2003; H. Tsuda et al., 2003; A.L. Bertone et al., 2004) or with nonviral vector (J. Bonadio et al., 1999; K. Honma et al., 2001; A. Sano et al., 2003; I. Ono et al., 2004). Here demonstrated is an *in vivo* gene transfer using nonviral vectors. This study was to examine whether our designed matrix, which consists of collagen, CaP, and a plasmid vector encoding for BMP2, enhances bone tissue regeneration in a rat bone defect model.

2.3.2 Materials and methods

Systemic administration of rhFGF4

Human FGF4 cDNAs vector were ligated to pET-29(+) vector (pET system, Novagen). After subcloning in JM109 and plasmid purification, the plasmids were transferred into BL21(DE3)pLysS, an E. coli strain used for protein expression. Protein expression was induced with isopropyl-b-Dthiogalactopyranoside (IPTG). The proteins were then purified using the STag Purification Kit (Novagen). The purified proteins were dialyzed against water using a minidialysis system (Bio-Tech International), and then freeze-dried.

Forty male ddY mice, 6 weeks old, were divided into eight groups and subcutaneously injected with rhFGF4s at doses of 0.03, 0.1, and 0.3 mg/kg every day for 2 weeks, which stimulated cellular proliferation of NIH3T3 cells, at doses of 0.03, 0.1, and 0.3 mg/kg. These rhFGF4s were dissolved in PBS containing 0.1% bovine serum albumin and injected. The five mice in the control group were injected with vehicle only.

After the 2 week injection course, the femurs were removed and contact microradiographs (CMRs) were taken of these ground sections using a soft X-ray radiographic apparatus (Sofron, SPO-M50).

For histomorphometric analysis, the sections were further ground down to 30 μm and stained with toluidine blue. Then, histomorphometric measurements were using an image analysis system (IBAS 2000, Carl Zeiss) to measure the histomorphometric parameters on the images of the areas. The measured parameters and the calculated parameters follow the previous report described in JBMR (A.M. Parfitt et al., 1987).

Local administration of rhFGF4

Thirty-two 10-week-old male Sprague-Dawley rats were divided into two equal groups; one group received local injection of rhFGF4 and the other received local injection of vehicle as control. An injection of 1 μg rhFGF4 (0.1 μg/μl) was given from the left tibial proximal intercondyler notch into the midshaft of the marrow cavity directly with a 21G needle.

The animals were killed under chloroform anesthesia on days 7 and 10. Tibiae were removed and photographed using soft X-rays. The bone mineral densities were measured with dual-energy X-ray absorptimetry. The trabecular and cortical bone marrow densities of the tibiae were measured with pQCT (Fig. 9A). After BMD measurements, the tibiae were fixed in 10% formalin, dehydrated, and embedded in methyl methacrylate resin (OsteoResin, Wako, Osaka, Japan). Then, longitudinal sections of 5-μm thickness were made via a microtome (Microtome 2050 Supercut, Reichert-Jung, Kandel Electronics, Inc., Oreland, PA) and stained by the Villanueva bone staining method. The x20 objectives of both light and fluorescence microscopes (Axiophot, Carl Zeiss, Oberkochen, Germany) were used to take optical images of the sections. Histomorphometric measurements were performed of the tibia using an image analysis system (IBAS 2000, Carl Zeiss).

BMP2 gene transfer at fracture site

cDNA of hBMP2 was inserted into pEGFP-N1 plasmid vector (Clontech, Mountain View, CA). hBMP2 encoding plasmid (bmp2) was precipitated in CaP solution (CalPhos Mammalian Transfection Kit, Clontech). An equal volume of 2% bovine type I atelocollagen solution (Atelocollagen Implant, Koken, Tokyo, Japan) was then added. Twelve micrograms of this mixture (50 μl) were lyophilized and designated "bmp2-CaP-collagen".

The bone segments across a 5-mm segmental tibial defect of male Wistar rats were fixed with stainless-steel screws. Implants were placed and held in the osteotomy. The specimens were fixed in 10% neutral formalin. Some specimens were embedded in methylmethacrylate resin and longitudinal sections that included the bone defects were then prepared. Undemineralized sections were stained with toluidine. For mechanical tests, the other samples were supported at the proximal and distal points on the jig, and force was applied to the middle of the bone defect perpendicularly at a displacement rate of 1 mm/min using a materials testing machine (Instron 1123, Cauton, MA). Force and displacement data were stored in the computer.

The sites of the bone defects were collected and homogenized immediately for total RNA extraction (Isogen, Nippon Gene, Tokyo, Japan). And then RT-PCR was performed (SuperScript First-Strand Synthesis System for RT-PCR, Invitrogen, Carlsbad, CA; PureTaq Ready-To-Go PCR Beads, Amersham Biosciences, Piscataway, NJ). Initially, denaturing was carried out at 95°C for 5 min, followed by optimizing cycles: 95°C for 30 s for denaturing, optimized temperature for 30 s for annealing, and 72°C for 30 s for extension. Each RT-PCR product was electrophoresed in 2% agarose gel in TAE buffer and stained with ethidium bromide, followed by photography under ultraviolet light.

2.3.3 Results

Systemic administration of rhFGF4

Soft X-ray images revealed an increase in trabecular bone was evident dose-dependently in the CMRs in the rhFGF4-administered group (Fig. 8).

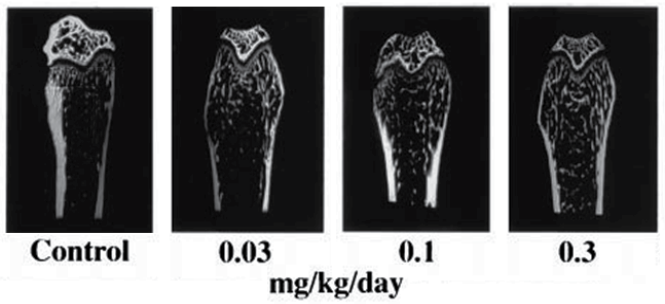


Fig. 8. Contact microradiographs (CMRs) of the longitudinal sections of femurs after administration with rhFGF4 (S. Kuroda et al., 1999).

Histomorphometric analysis revealed an increase in BV/TV and Tb.N, which represents an increase in trabecular bone. Furthermore, bone formation parameters (MS/BS and OS/BS) increased in a dosedependent manner, whereas a bone resorption parameter (ES/BS) was not affected (Table 1).

	rhFGF4 (mg/kg)			
	0 (Control)	0.03	0.1	0.3
BV/TV (%)	18.3 ± 2.5	23.4 ± 1.9	28.9 ± 1.5	31.1 ± 2.3
ES/BS (%)	10.5 ± 1.2	9.0 ± 1.6	9.8 ± 0.9	11.8 ± 1.1
MS/BS (%)	18.5 ± 2.7	20.0 ± 2.7	39.4 ± 2.5	43.1 ± 2.5
OS/BS (%)	17.5 ± 1.0	19.0 ± 2.6	30.4 ± 2.1	42.2 ± 1.9
Tb.Th (μm)	30.9 ± 1.3	28.8 ± 1.5	30.1 ± 2.3	33.7 ± 2.3
Tb.N (μm)	2.7 ± 0.2	3.0 ± 0.1	3.8 ± 0.2	3.8 ± 0.2

Table 1. The increase of trabecular bone after administration with rhFGF4 was measured and confirmed with histomorphometric parameters. Data are presented as the mean ± SE (n=5). a: Significantly different from controls, $p<0.05$. (S. Kuroda et al., 1999)

Local administration of rhFGF4

There were no visible or weight differences in the rats between the 2 groups at each time point, and neither the shapes nor sizes of the tibiae were affected by the local injection of rhFGF4 (data not shown). However, soft X-ray images demonstrated less radiolucence in the rhFGF4 group (Fig. 9A). DEXA analysis revealed increased BMD of the cancellous bone-rich zone of tibiae after the local injection of 1.0 mg of rhFGF4 and significance between the 2 groups at day 10 (Fig. 9B). Similarly, based on pQCT analysis (Fig. 9C), the trabecular BMD increased significantly in the rhFGF4 group from day 7 to day 10. Further, the higher BMD was maintained by the rhFGF4 injection over time. On the other hand, the cortical BMD exhibited no difference either between the groups or over time (data not shown).

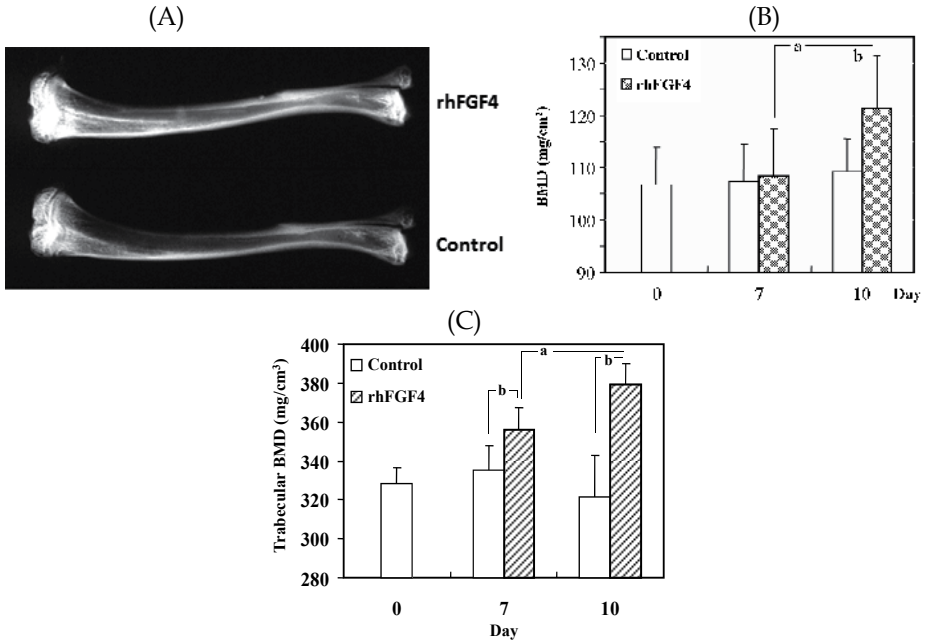


Fig. 9. (A) Soft X-ray images of the tibiae. (B) BMD of the tibiae by DEXA. (C) Trabecular BMD of the mid shafts of the tibiae by pQCT. [a] Significantly increased by time, [b] Statistically different between the groups, $p < 0.05$. (S. Kuroda et al., 2007)

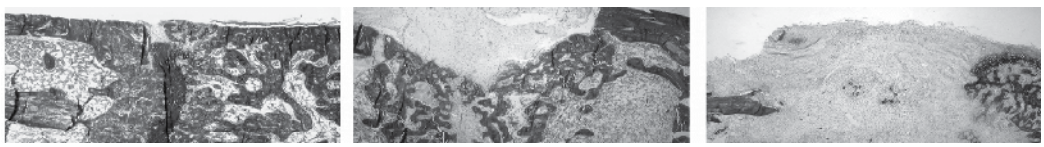
Histomorphometric analysis elucidated increases in BV/TV, OS/BS, Ob.S/BS, MS/BS, ES/BS and Oc.S/BS in the rhFGF4 group at day 7 (Table 2), which represents high turnover of bone remodelling and derived increase of trabecular BMD. However, the ratios of parameters to BS were decreased at day 10; in particular, the ratios of OS, Ob.S, ES and Oc.S to BS were significantly decreased from day 7.

	Day 7		Day 10	
	Vehicle	rhFGF4	Vehicle	rhFGF4
BV/TV	15.3±6.7	23.9±3.8	15.0±5.6	30.6±7.8*a*
OS/BS	16.5±3.1	29.3±3.5*a*	13.4±1.8	17.5±1.6*b*
MS/BS	18.6±3.0	31.7±2.5*a*	12.6±0.7	23.6±7.2*a*
ES/BS	16.6±4.0	23.5±1.4*a*	10.4±1.8	16.2±1.1*a*
Ob.S/BS	10.7±3.4	24.4±4.7*a*	10.3±1.8	14.1±2.0*b*
Oc.S/BS	10.6±2.9	22.3±2.8*a*	11.2±2.4	14.1±3.3*b*
Tb.Th	22.5±9.6	26.3±9.9	22.9±6.9	28.7±7.8
Tb.N	6.6±0.8	9.6±2.2	5.6±0.6	11.3±2.3*a*

Table 2. Histomorphometry was analysed with following parameters. BV: bone volume; TV: tissue volume; BS: bone surface; OS: osteoid surface; MS: mineralized surface (single labeled by calcein); ES: eroded surface; Ob.S: osteoblast surface; Oc.S: osteoclast surface; Tb.Th: trabecular thickness; Tb.N: trabecular number. Data represents the mean of 4 samples from the both groups ± SD. a indicates values that are significantly different from control, $p < 0.05$. b indicates values that become significantly different in the same group, $p < 0.05$. (S. Kuroda et al., 2007)

BMP2 gene transfer at fracture site

Histological examinations indicated that the group treated with bmp2-CaP-collagen showed the most abundant osteogenesis (Fig. 10). The osteotomy site was connected with fibrous tissue only at 2 weeks. It was connected with callus and the gap was filled with newly formed cartilage and bone at 4 weeks after the operation. At 6 weeks, although there were still remnants of cartilage in the center, newly formed bone was remodeled to the cortical or cancellous bone and fused to the stump of the host bone, and the osteotomy sites became unclear. When treated with bmp2-collagen, the bone defect was bridged at 6 weeks but the bridged area was smaller and newly formed bone was less mature than that of the bmp2-CaP-collagen group. In the group treated with only collagen, a residue remained at 6 weeks, and although small callus formation from the host bone was observed, the defects were mainly filled with fibrous tissue.



bmp2-CaP-collagen **bmp2-collagen** **collagen**

Fig. 10. Histological images of the defects treated with different implants (M. Endo et al., 2006).

Longitudinal sections were prepared at 6 weeks after the operation. Sections were stained with toluidine blue (original magnification x 40).

Mechanical strength to fracture at the osteotomy sites is presented in Fig. 11. The groups treated with bmp2 and collagen could be measured; however, the single implant of collagen did not induce the bone bridge. At 4 weeks, the bone treated with bmp2-CaP-collagen was stronger than that treated with bmp2-collagen. The mechanical strength of bone treated with bmp2-CaP-collagen was closely similar to that of the contralateral tibia at 6 weeks. In this group the fracture did not occur at the osteotomy site, but at the host bone.

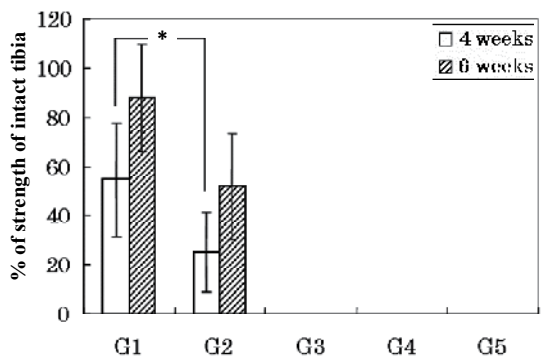


Fig. 11. Mechanical strength of the tibiae treated with different implants (M. Endo et al., 2006).

Values are presented as mean ± standard deviation. The statistically significant difference is observed between G1 and G2 at 4 weeks ($p<0.05$). G1: bmp2-CaP-collagen; G2: bmp2-collagen; G3: collagen.

Both rat and human BMP2 gene expression were detected throughout the experimental period in Fig. 12: human BMP2 gene expression level did not alter so much with time, and, similarly, the expression level of rat BMP2 gene remained up to 8 weeks. The expression level of osteocalcin gene was elevated up to 8 weeks. On the other hand, VEGF genes were evenly expressed during the period. RANK and RANKL gene expression were enhanced initially, and the levels were maintained until 8 weeks.

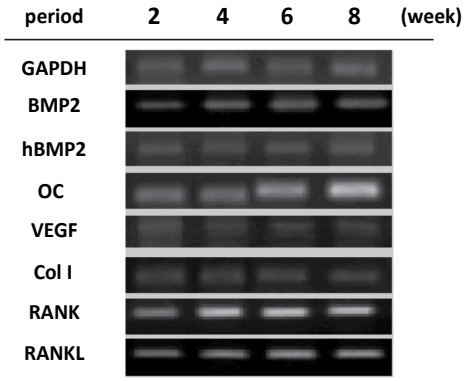


Fig. 12. Gene expression at the defect site treated with bmp2-CaP-collagen (M. Endo et al., 2006). One to five μg of total RNA of each sample was reverse-transcribed to cDNA in 20 μl, 1 μl of which was used for PCR amplification in 50 μl. And then 8 μl out of 50 μl was run for a gel electrophoresis.

3. Discussion and conclusion

OVX

In OVX rats, both bone formation and resorption is accelerated; however, the unbalance, more bone resorption than bone formation, causes trabecular bone loss in long bones and vertebrates (Y. Otawara et al., 1983; T.J. Wronski et al., 1989a, 1989b; B.C. Toolan et al., 1992). In the present study, total femoral BMD of OVX rats decreased in DEXA analysis. This BMD decrease in OVX rats was due to the decrease in trabecular bone and cortical bone was not affected, which was revealed in pQCT analysis. These results are the same as previously reported by other investigators (P. Pastoureau et al., 1995; C.M. Bagi et al., 1996; S.A. Breen et al., 1996).

Firstly, BMD measurement of condylar region demonstrated BMD decrease in OVX rats, minus 14% from BMD of shame-operated one. Percent BMD decrease of this region was similar to the decrease of femoral BMD in OVX rats. Secondary, BMD of trabecular bone of molar region of mandible decreased in OVX rats, which was revealed in pQCT analysis. Notably, the extent of this BMD decrease (maximal 13% decrease) was less than the decrease of trabecular bone of the femur in OVX rats (30% decrease at the 17-mm-section). Thus, the

susceptibility of the bone of the molar region of the mandible to estrogen deficient condition was low compared to the bones of condylar region of the mandible and the femur.
In the present study, we found trabecular bone decrease of the molar region of the mandible in OVX-rats. Although the mechanism of the low susceptibility of the molar region of the mandible to estrogen deficient condition is not clear, it is likely that mechanical stress derived from functional occlusion is preventing the bone loss in this pathological condition. Elovic and his collaborators have clearly demonstrated that maxillary molar extracion together with ovariectomy causes more bone loss in the mandible than maxillary molar extraction alone (E. Klemetti et al., 1993a, 1993b, 1994; R.P. Elovic et al., 1994; E. Klemetti & P. Vainio, 1994; R.P. Elovic et al., 1995; L. Jahangiri et al., 1997), which supports this speculation.

GBR

Cell migration is likely influenced by the size of the inter-fibrous space in the molecular construction of the membranes (J. Behring et al., 2008). As such, cells were able to migrate inside the collagen membrane. The collagen was found to perform well as a new bone space maintainer excluding the adjacent soft tissue. Several cell-culture studies have explored the biocompatibility of GBR membranes by comparing their levels of inflammatory-related gene expression (A. Friedmann et al., 2008) and osteogenic markers (S.B. Idris et al.). However, only a few *in vivo* researches before this study had done so by developing and comparing gene expression profiles rather than performing radiological and histological analysis during osteogenesis in the bone defect (M. Nyan et al.). By allowing for observation of differences in cellular events between the experimental (with membrane) and control (no membrane) conditions, the development and comparison of gene expression profiles will permit molecular examination of the bone-healing process in the future study.

Systemic/local administration of rhFGF4

The most characteristic feature of the systemic effects of FGF4 is likely the stimulation of endosteal but not periosteal bone formation. This is elicited by proliferation of preosteoblastic cells in bone marrow, followed by recruitment of osteoblasts from preosteoblastic cells (T. Nakamura et al., 1995; S. Kuroda et al., 1999). Subsequently, the increase of cancellous bone becomes prominent (H. Mayahara et al., 1993; T. Nakamura et al., 1995). As suggested, FGF family members are of great importance for bone development and morphogenesis (B. Feldman et al., 1995; H. Ohuchi et al., 1995; R.A. Buckland et al., 1998). Further, the expression of FGF family members such as basic FGF is often upregulated during fracture repair and may contribute to the regeneration process (M.E. Bolander, 1992). Some reports have suggested that exogenous FGF family members accelerate bone fracture healing and wound healing when locally applied (H. Kawaguchi et al., 1994; T. Nakamura et al., 1998) and also recover bone mass that has been pathologically damaged because of ovariectomy or diabetes, for instance (H. Kawaguchi et al., 1994; C.R. Dunstan et al., 1999). Therefore, although FGF4 has not been detected in postnatal stages, it is assumed that exogenous FGF4 during tissue regeneration might have important aspects as well as basic FGF or other growth factors (R.K. Globus et al., 1989; M. Noda & J.J. Camilliere, 1989; M.E. Joyce et al., 1990; M.E. Bolander, 1992), and exogenous FGF4 as well as basic FGF may possibly enhance the local regeneration (M. Noda & R. Vogel, 1989).

The ratio of bone volume to tissue volume indicated that new bone formation seemed to have started before day 7 and this value became significantly higher on day 10 than that in the control group. The changes of these histomorphometric parameters might be attributed to an increase in the endosteum bone callous, which was supported by a prominent increase in the affected trabecular BMD but not the cortical BMD. Thus, rhFGF4 serves as an anabolic molecule in bone.

These studies were designed to analyze changes of bone mineral density or content with DEXA under osteoporosis, external growth factor intake and GBR in a skeletal defect, respectively. DEXA is widely used for obtaining an averaged mineral density of each part of a sample by directional scanning in as narrow a width as approximately 1 mm. These averages can be sammed for the total bone area to observe the total mineral content and density. The densities are expressed in a unit of mg/cm^2 because the bone, originally three-dimensional, is recognized as a flat picture during the scanning process. While pQCT and micro computed tomography (μCT) are provided for three-dimensional analyses of bone density and/or digital reconstructing of bone for several indices of morphometry, DEXA allows much easier and faster settings of samples and calculating of densities in resions of interest (ROI). Therefore, the key point and the characteristics of DEXA are giving an initial idea to get to know time-dependent changes of a sample or difference among samples. Overall, DEXA, pQCT and μCT can provide convenient and prompt tools, which can perform acculate comparisons.

Gene trasnfer for BMP2

There are three phases in the bone regeneration process: 1) the early inflammatory phase; 2) the repair phase; and 3) the remodeling phase (V.I. Sikavitsas et al., 2001). In the early inflammatory phase, the hemorrhage and the subsequent hematoma are followed by infiltration of inflammatory cells and fibroblasts to the repair area. These events lead to vascularization and the formation of granulation tissue. The second phase is the repair phase, which is characterized by a callus. This phase begins with vascular ingrowth, osteoid secretion and the presence of collagenous fibers. A temporary callus consisting of cartilage is produced. In the remodeling phase, osteoblasts are active and the cartilage tissue is replaced with immature cancellous bone. Some cancellous bone is then converted to mature dense bone. Although histological examination was performed at the limited time points in the present study, it is likely that these sequential events occurred in the present bone defect. This repair process was clearly modified by the different combinations of implanted materials.

Constant expression of VEGF indicates importance of neovascularization during the healing process. Since RANK, receptor activator for NFκB on the cellular membrane of osteoclast progenitors and osteoclasts, and its ligand RANKL, which is secreted from osteoblasts, are involved in osteoclast maturation and activation, maintained RANK expression suggests onward activation of osteoclasts leading to the bone remodeling including the callus. On the other hand, osteocalcin is a bone-specific matrix protein which is produced by mature osteoblasts. Thus, the increase of osteocalcin gene expression at 6 weeks implicates reaching the maturation of the defect site. Although rat BMP2 gene as well as human BMP2 gene diminished gradually, the gene expression remained detected over the period, suggesting enhanced osteoblast incorporation into the fractured site.

The mechanism of how the GAM system stimulates tissue regeneration is speculated as the following (J. Fang et al., 1996; J. Bonadio et al., 1999, 2000). CaP, in which plasmid vector is incorporated, has been used for *in vitro* gene transfer (C.F. Graham, 1973; A. Loyter et al., 1982a, 1982b; S. Kato et al., 1986; E. Orrantia & P.L. Chang, 1990; M. Werner et al., 1990; A.V. Zelenin et al., 1991; J.K. Burkholder et al., 1993; J.C. Sanford et al., 1993; T.A. Thompson et al., 1993). Since CaP stabilizes nucleic acids (A. Loyter et al., 1982a, 1982b), we speculated that CaP would be also useful for *in vivo* gene transfer (Y.W. Yang & J.C. Yang, 1997; S.Y. Watanabe et al., 1999; P. Batard et al., 2001). In the regeneration process, the cells surrounding GAM migrate into the matrix. Fundamentally, it is likely that the cells migrating into GAM are mainly fibroblastic cells and some of these cells can be regarded as targets for the plasmid. They engulf the plasmid vector, subsequently starting to produce an encoded protein. Thus, GAM acts as a bio-reactor for producing an encoded protein, which was human BMP2 in the present study. After transplanting our modified GAM, human BMP2 gene expression was observed at almost the same level throughout the experimental period. It is clear that human BMP2 produced by transfected cells enhanced bone regeneration in the present study. The duration of the gene expression in this gene transfer system depends on the site of the application and the size of the GAM, which presumably influences the period of the matrix degradation and the duration of gene expression. Although we did not observe cessation of human BMP2 gene expression, the expression of human BMP2 gene declined until time suggesting temporality of the expression of the transfect gene.

The results of the radiographic and histological analyses demonstrated that this critical size bone defect was bridged when it was treated with bmp2 and collagen. Notably, the regeneration of the defects treated with bmp2-CaP-collagen was more prominent than that of the defects treated with bmp2-collagen. These results were also confirmed in the biomechanical test.

Ideas to reduce and avoid the emergence of compromised bone status such as osteoporosis, fractures and critical skeletal defects, and to increase bone mineral density and bone volume must be a theme for minimizing the burden of fractures through interventions that help to achieve optimal peak bone mass, reduce excessive skeletal resorption, enhance bone formation.

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Lactoferrin – A Potential Anabolic Intervention in Osteoporosis

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1. Introduction

Osteoporosis or porous bone was first described by Fuller Albright approximately 70 years ago as having "too little bone in the bone". Bone tissue is maintained throughout life by being continually replaced and in osteoporosis bone resorption exceeds bone formation resulting in bone loss. The majority of current treatments for osteoporosis are anti-resorptive, decreasing osteoclast activity and preventing further bone loss. Therapeutic agents that activate osteoblasts and increase bone formation have the potential benefit of restoring bone rather than only preventing further deterioration, but only a small number of safe anabolic therapies are currently available. Milk is a rich biological fluid that contains many growth factors and provides nutrition at a time of very rapid skeletal growth and development in the neonate, and was therefore considered as a possible source of factors with anabolic effects on bone. Investigations of fractions of whey proteins extracted from milk identified lactoferrin as a bone-active factor. Lactoferrin is an iron-binding glycoprotein which as well as being present in milk is found in other epithelial secretions. It is a pleiotropic factor with potent antimicrobial and immunomodulatory activities, and shows anabolic effects in bone at physiological concentrations. In a number of recent studies in humans and experimental animals dietary lactoferrin supplementation improved bone mineral density, bone markers and bone strength. The current chapter discusses the structure and function of lactoferrin, the bone-effects of lactoferrin *in vitro* and *in vivo*, and the potential use of lactoferrin for the improvement of bone health.

2. Lactoferrin

Lactoferrin is a multifunctional glycoprotein that was originally identified in bovine milk and first isolated from both human and bovine milk five decades ago (Groves et al. 1963). Lactoferrin is produced by mucosal epithelial cells and is present in very high concentrations in milk and colostrum, and in lower concentrations in mucosal secretions, including tears, saliva, nasal and bronchial secretions, bile and gastrointestinal fluids. Lactoferrin is also a major constituent of the secondary granules of neutrophilic leukocytes, and its serum level in healthy subjects is within the range of 1-10 μg/mL (Caccavo et al. 1999).

2.1 Molecular structure

Lactoferrin is a non-haem iron-binding protein which belongs to the transferrin family of iron-transport proteins. It is a highly cationic monomeric glycoprotein with an isoelectric point of about 8.7 (Moguilevsky et al. 1985) and consists of about 690 amino acid residues folded into two homologous lobes, the so-called N-and C-lobes, which are linked by a 10-15 residue alpha helical peptide (Baker & Baker 2005). There is a high degree of homology between lactoferrin from various species with bovine and human lactoferrin sequence identity being 69% (Pierce et al. 1991).

2.1.1 Iron binding and glycosylation

Each of the lactoferrin lobes contains a virtually identical iron-binding pocket, into which a trivalent iron cation (Fe3+) can be reversibly co-ordinated. The metal binding sites are highly conserved for all lactoferrins and transferrins thus far characterised (Baker & Baker 2009). Lactoferrin molecules can exist in several states whereby there is complete, partial (in either one of the two sites) or no occupancy of the two iron-binding sites. Lactoferrin isolated from both human and bovine milk has a low iron saturation, generally reported between 10-25% (Bezwoda & Mansoor 1989). In vitro, iron can be removed from lactoferrin to yield the iron-free or 'apo' form, or alternatively, lactoferrin can be loaded with iron to yield the fully iron-bound or 'holo' form. Although other di- and trivalent transition metal ions such as Mn3+, Co3+, Cu2+ and Cr 3+ and even larger cations such as lanthanides (Smith et al. 1994) can be co-ordinated into the metal binding pocket, iron appears to be the natural ligand as it has optimal co-ordination and a very high binding affinity (Baker 1994). Nevertheless, lactoferrin may have a physiological role in binding trace amounts of other elements as manganese in milk is found exclusively associated with lactoferrin (Lonnerdal et al. 1985).

All lactoferrins are glycosylated, but the number and location of glycosylation sites varies from species to species, and is also tissue specific (Derisbourg et al. 1990). Differentially glycosylated lactoferrins appear to have similar biophysical and functional properties suggesting minimal structural impact of glycosylation (Moguilevsky et al. 1985).

2.1.2 Interactions with other molecules

The highly basic nature of lactoferrin is contributed mainly by surface-exposed N-terminal domains containing clusters of highly basic residues which are capable of binding proteins such as ceruloplasmin (Vasilyev 2010) and osteopontin (Yamniuk et al. 2009). These cationic domains also confer on lactoferrin the ability to bind to many other anionic molecules including heparin, glycosaminoglycans, DNA, and various cell surface molecules (He & Furmanski 1995; Mann et al. 1994; van Berkel et al. 1997).

2.2 Physiological function

2.2.1 Anti-microbial activity

The highly cationic nature of lactoferrin and its high affinity iron binding are implicated in the anti-microbial function of this glycoprotein. Thus, iron sequestration in sites of bacterial infection deprives the bacteria of this essential nutrient, creating a bacteriostatic effect (Gonzalez-Chavez et al. 2009; Jenssen & Hancock 2009). Lactoferrin has bactericidal effect as well, as it interacts directly with anionic molecules on the cell surface, causing cell lysis. In Gram-negative bacteria, lactoferrin interacts directly with LPS, causing its release from the cell wall and increasing the external membrane permeability, which results in cell lysis. In

Gram-positive bacteria lactoferrin damages the cell through direct interactions with lipoteichoic acid and other anionic surface molecules. Iron sequestration and interactions with anionic molecules are also the main mechanisms responsible for lactoferrin's activity against fungus and parasite infections. Lactoferrin has been described as an antiviral agent that affects a broad range of RNA and DNA viruses that infect humans and animals (Gonzalez-Chavez et al. 2009). Although the antiviral mechanisms of lactoferrin have not been well characterised yet, one of the most widely accepted hypothesis is that lactoferrin blocks viral receptors on the cell surface, and in particular heparin sulphate, preventing contact between the virus and the target cell.

2.2.2 Modulation of the immune response and inflammation

Lactoferrin is a modulator of both the innate and acquired immune systems. Following the penetration of a microbe into a tissue, cells of the innate immune system release pro-inflammatory cytokines, including interleukins 1 and 6 (IL-1, IL-6) and tumor necrosis factor-alpha (TNF-α), which increase the permeability of blood vessels enabling the recruitment of circulating neutrophils to the site of infection. The release of neutrophil granule content creates very high local concentrations of lactoferrin. Apart from direct antimicrobial activity, lactoferrin interacts with cells of the innate immune systems as well as with cells of the adaptive immunity; regulating their recruitment, proliferation, differentiation and activation (Legrand et al. 2006; Legrand & Mazurier 2010).

In different experimental systems, lactoferrin acts as either an anti-inflammatory or a pro-inflammatory factor. The anti-inflammatory activity of lactoferrin is attributed to its ability to bind free iron and exogenous proinflammatory bacterial components, such as LPS and their receptors (Legrand et al. 2005). Thus, lactoferrin activity as an iron scavenger prevents the formation of free radicals, which trigger oxidation processes and tissue damage, while binding to proinflammatory molecules inhibits the activation and recruitment of immune cells to the inflamed tissue. An additional mechanism implicated in the anti-inflammatory activity of lactoferrin has been recently described in apoptosis, the process of noninflammatory programmed cell death. Bournazou et al. (Bournazou et al. 2009; 2010) discovered that apoptotic cell of diverse lineages synthesize and secrete lactoferrin, which selectively inhibits the migration of granulocytes but not mononuclear phagocytes. This selective migration allows for the swift phagocytosis of the dying cells by the mononuclear cells without initiating an inflammatory response. Subsequently, lactoferrin was also found to have an inhibitory effect on eosinophil migration (Bournazou et al. 2010). A number of other studies describe the proinflammatory activities of lactoferrin. As a factor that induces inflammation, lactoferrin has been shown to promote cell motility, superoxide production, release of nitric oxide, release of the proinflammatory cytokines TNF-α and IL-8 and phagocytosis (Gahr et al. 1991; Legrand & Mazurier 2010; Shinoda et al. 1996; Sorimachi et al. 1997).

2.2.3 Iron homeostasis and antioxidation

Although lactoferrin is an iron-binding protein and has been shown to influence iron status in pregnant women (Paesano et al. 2009) it is generally thought not to have a central role in iron-transport and homeostasis, unlike the transferrins. Nevertheless it does appear to have some role in iron regulation at local sites of inflammation and infection (Brock 2002) and

iron sequestration is an important part of its role both as an antioxidant and antibacterial agent (Baldwin et al. 1984; Jenssen & Hancock 2009). In respect of iron-binding, lactoferrin is functionally different from transferrin as it retains iron to a much lower pH, giving it a more potent iron-withholding ability (Baker & Baker 2009). Although the antioxidant properties of lactoferrin are generally related to removal of free iron which otherwise reacts with reactive oxygen species (ROS) to form damaging hydroxyl radicals (Matsue et al. 1995; Raghuveer et al. 2002) a recent report suggests that apo- and holo bovine-lactoferrin have equal ability to act as antioxidants by scavenging ROS (Kanwar et al. 2011). This radical quenching ability, akin to antioxidant vitamins, is seemingly iron independent.

2.2.4 Bioactive peptides derived from lactoferrin

Functional cationic peptides with potent antibacterial activity, such as lactoferricin and lactoferrampin, can be derived from the N-terminal domain of lactoferrin by hydrolysis (Bellamy et al. 1992) or synthetic chemistry (van der Kraan et al. 2004), respectively. Lactoferrin can be degraded by digestive enzymes (Brock et al. 1976; Troost et al. 2001) and the functional peptide lactoferricin is likely to be formed in the gut by the action of pepsin. Lactoferrin 'half molecules' consisting of either the N-lobe or C-lobe can be generated by proteolysis or by recombinant technology (Baker & Baker 2005; Kim et al. 2006). These are useful as tools to probe for site-specific functionality or interactions. For example, the anti-herpes virus activity of lactoferrin has been shown to be mediated mainly by the N-lobe (Siciliano et al. 1999) while simple sugars have been shown to interact with the C-lobe through a common recognition site (Mir et al. 2010).

3. The activity of lactoferrin in bone

3.1 Osteoblasts

3.1.1 *In vitro* studies of lactoferrin activity in osteoblasts

Lactoferrin potently induces proliferation of primary osteoblasts and osteoblastic-cell lines and increases osteoblast differentiation at physiological concentrations (Fig 1A) (Cornish et al. 2004; Takayama & Mizumachi 2008, 2009). In 3-week cultures of primary fetal rat osteoblasts lactoferrin dose-dependently increased osteoblast differentiation with increases in bone matrix deposition and the number of mineralized bone nodules formed (Fig 1B) (Cornish et al. 2004). In addition, lactoferrin decreased apoptosis induced by serum withdrawal in primary rat osteoblasts (Fig 1C) (Cornish et al. 2004) and in the human osteoblastic cell line SaOS2 (Grey et al. 2006). These effects on both the proliferation and survival of osteoblasts are profound, being far greater than those observed in response to several established osteoblast growth factors studied in the same *in vitro* assays, such as epidermal growth factor, transforming growth factor-β, parathyroid hormone, amylin or insulin. These factors increase thymidine incorporation in primary osteoblast cultures by only 20 – 30% (Cornish et al. 1999) whereas lactoferrin produces three- to five-fold increments (Cornish et al. 2004). This growth stimulating potency is complemented by the capacity of lactoferrin to substantially reduce osteoblast apoptosis, which again, is much more dramatic than the effects seen with other factors, such as insulin growth factor-1 (IGF-1) which maximally decreases apoptosis by 50% (Cornish et al. 2000) compared to 70% with lactoferrin (Cornish et al. 2004).

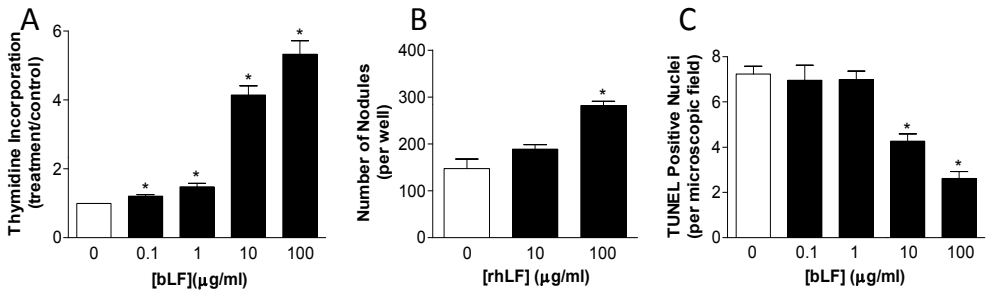
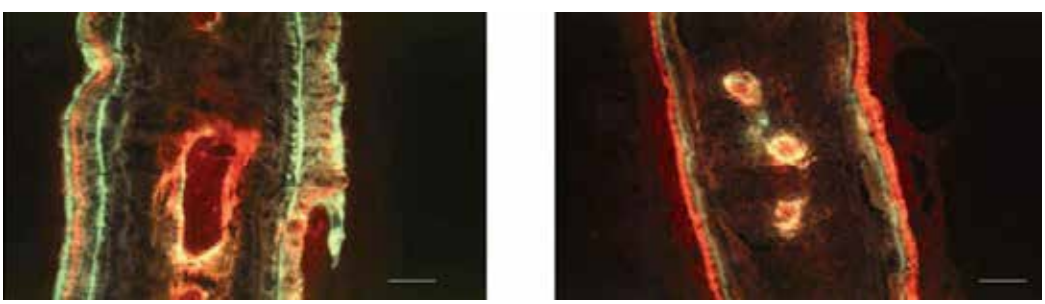


Fig. 1. Lactoferrin stimulates osteoblast proliferation, differentiation and survival *in vitro*

Thus, lactoferrin acts to expand the pool of pre-osteoblastic cells by exerting mitogenic and anti-apoptotic effects, as well as promoting differentiation of precursors to produce a more mature osteoblastic phenotype capable of promoting bone matrix deposition and mineralization.

3.1.2 Local injection model

The activities of lactoferrin on osteoblasts demonstrated *in vitro* are likely to contribute to the potent effects on bone formation seen *in vivo* after administration of lactoferrin, even with a very short-term exposure (Fig 2) (Cornish et al. 2004). The bone growth resulting from local lactoferrin injection is considerably greater than that found previously in response to factors such as insulin, amylin, adrenomedullin and C-terminal PTH-related peptide (Cornish et al. 1996; 1997a; 1997b). It is qualitatively different from the effects of PTH in this model, which produces a powerful stimulation of bone resorption in addition to its effect on formation (Cornish et al. 1995). This anabolic potency suggests that lactoferrin should be further explored as a therapy for osteoporosis that can restore skeletal strength.



A B

Fig. 2. Photomicrographs of calvariae from animals treated with lactoferrin (A) and vehicle (B) for 5 days. Fluorochrome labels used: green, calcein; red, alizarin. Horizontal bar, 50 μm. (Figure reproduced with kind permission. Cornish J., et al. Lactoferrin is a potent regulator of bone cell activity and increases bone formation in vivo. Endocrinology 145(9): 2004, 4366-4374. Copyright 2004, The Endocrine Society.)

3.1.3 Signalling pathways activated by lactoferrin in osteoblasts

The downstream pathways activated by lactoferrin are largely unknown, although a number of lactoferrin receptors have been described. A specific lactoferrin receptor was cloned from the human intestine (Kawakami & Lonnerdal 1991) but this receptor is not expressed in all cell types that respond to lactoferrin and we have been unable to detect the mRNA in osteoblastic cells (Naot, unpublished data). Proteins that can bind and induce endocytosis of lactoferrin are nucleolin (Legrand et al. 2004) as well as low-density lipoprotein receptor-related proteins 1 and 2 (LRP1 and LRP2) (Ji & Mahley 1994; Willnow et al. 1992). LRP1 and LRP2 are expressed in osteoblastic cells and LRP1 is at least partially responsible for lactoferrin's mitogenic effects in osteoblasts (Grey et al. 2004). As lactoferrin complexes with LRP1, extracellular signal-regulated kinase (ERK) signalling pathway is upregulated. In addition, lactoferrin upregulates phosphoinositide 3-kinase-dependent Akt signalling but this is in an LRP-independent manner. Lactoferrin's anti-apoptotic activity in osteoblasts is independent of both these two signalling pathways.

In primary osteoblasts, lactoferrin induces a transient, dose-dependent increases in the transcription levels of IL-6, IL-11, the pro-inflammatory factor prostaglandin-endoperoxide synthase 2 (Ptgs2, encoding for the enzyme cyclooxygenase-2, COX-2) and the transcription factor nuclear factor of activated T-cells (Nfatc1). The activity of COX-2 to produce and secrete prostaglandin E2 and the activity of NFATc1 to promote transcription from NFAT consensus elements are also induced by lactoferrin. Moreover, COX-2 and NFATc1 act as mediators of the proliferative effect of lactoferrin in osteoblasts, as inhibition of their activities significantly reduces lactoferrin-induced thymidine incorporation (Naot et al. 2011). Recently, Nakajima et al demonstrated that lactoferrin induces synthesis of angiogenic factors by osteoblasts. In murine osteoblast-like MC3T3-E1 cells and primary murine osteoblasts lactoferrin, purified from milk, increased mRNA expression of vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF2) in a p44/p42 MAP kinase-dependent manner (Nakajima et al. 2011). A summary of some of the signalling pathways'activated by lactoferrin in osteoblasts is presented in Figure 3.

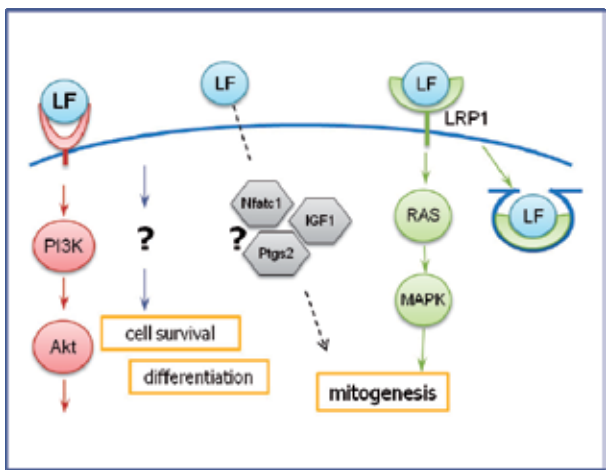


Fig. 3. Mechanisms of action of lactoferrin in osteoblasts. Figure reproduced with kind permission from Springer Science+Business Media: (*Biometals,* Lactoferrin as an effector molecule in the skeleton, 23, 2010, 425-430, Cornish, J. & Naot, D. Figure 1.)

3.1.4 Lactoferrin effects on early stages of osteogenic differentiation

Lactoferrin supports osteogenic differentiation in mesenchymal pluripotent cells whilst reducing adipogenic differentiation. In the pluripotent mesenchymal cell line, C2C12, analyses of expression levels of mRNA and proteins indicated an induction of osteoblastic and chondroblastic differentiation markers and a reduction in myoblastic and adipocytic markers (Yagi et al. 2009). We have identified that lactoferrin reduces adipogenic differentiation in KUSA4b10 cells, a mouse mesenchymal progenitor cell-line capable of developing into adipogenic or osteogenic cell lineages (Fig 4). Lactoferrin has also been found to promote the proliferation and osteogenic differentiation of human adipose stem cells (Ying et al. 2011). The activity of lactoferrin to support osteogenic differentiation whilst reducing adipogenic differentiation could be a promising approach for enhancing osteogenic capacity of cell-based construction in bone tissue engineering.

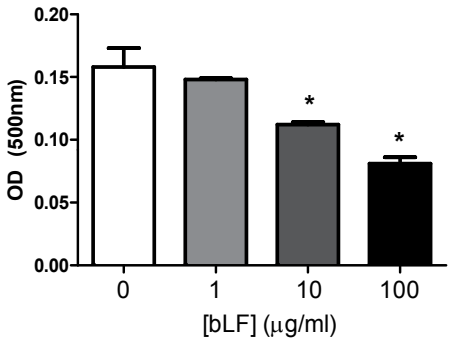


Fig. 4. Lactoferrin reduces adipogenic differentiation in KUSA4b10 cells as measured by Oil Red O release detected in a spectrophotometer at an optical density (OD) of 500nm.

3.2 Osteoclasts

In comparison with actions of lactoferrin on osteoblasts, its osteoclasts effects are strikingly different, in that it produces an almost total arrest of osteoclastogenesis in mouse bone marrow cultures (Fig 5) (Cornish et al. 2004). Reduced bone-resorbing activity was also demonstrated by Lorget et al, who used bovine lactoferrin in a rabbit mixed bone cell culture (Lorget et al. 2002). The mechanisms implicated in the inhibitory effect of lactoferrin on bone resorption are only partially understood. In the rabbit bone cell cultures, lactoferrin inhibited the development of mature osteoclasts by a mechanism independent of the receptor activator of NF-κB (RANK)/RANK-ligand (RANKL)/osteoprotegerin (OPG) system. In the mouse bone marrow cultures, lactoferrin reduced RANKL expression, which could in part explain the inhibition of osteoclastogenesis, although this was counterbalanced by the effects of lactoferrin to also inhibit expression of OPG (Cornish et al. 2004). As the RANK/RANKL/OPG pathway does not appear to play a major role in mediating the inhibitory effect of lactoferrin on osteoclast formation, the possibility of a direct effect on osteoclasts has been investigated. In RAW264.7 cells, which differentiate into osteoclasts in vitro in the absence of osteoblasts or stromal cells, lactoferrin inhibited RANKL-induced osteoclastogenesis in a dose-dependent manner, demonstrating a direct effect on osteoclasts (Cornish & Naot 2010). This effect of lactoferrin was not blocked by an inhibitor of LRP1, indicating that LRP1 is not the receptor that mediates the direct inhibition of

osteoclastogenesis by lactoferrin (Cornish & Naot 2010). It should be noted that lactoferrin has a capacity to inhibit the survival of progenitor cells in the bone marrow, implying that it might also act earlier in osteoclast development (Hangoc et al. 1991). In contrast to its inhibitory effect on osteoclast development, lactoferrin had no effect on bone resorption by isolated mature osteoclasts nor in organ cultures which also detect mature osteoclast activity (Cornish et al. 2004). Thus, lactoferrin inhibits bone resorption by reducing the number of osteoclasts formed from precursor cells.

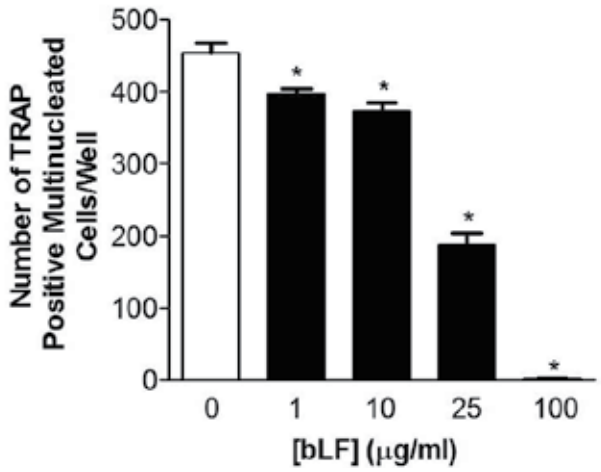


Fig. 5. Inhibition of osteoclastogenesis by lactoferrin in mouse bone marrow cultures

3.3 Structure/Function relationship of lactoferrin's bone activity

Bovine, human and recombinant forms of lactoferrin have comparable ability in stimulating osteoblast proliferation (Cornish et al. 2004). This suggests that glycosylation is not critical to the mitogenic activity as these three forms of lactoferrin are differentially glycosylated. Furthermore, when carbohydrate chains were removed from bovine lactoferrin, the aglyco-form was as potent as the glyco-form (Cornish et al. 2006), confirming that carbohydrate is not a major determinant in the mitogenic activity of lactoferrin in osteoblasts. The activity of lactoferrin on bone cells was also shown to be independent of iron-binding with apo-, native and holo- preparations of bovine lactoferrin giving similar levels of stimulation of proliferation (Cornish et al. 2006). Moreover, replacement of the iron with chromium and manganese, two transition metals of equivalent size, also had no effect. This suggests firstly that the conformational changes induced by iron-binding have no impact on lactoferrin activity, and secondly that bound iron is not essential to this activity.

Further structure/function studies were performed using the N-lobe and C-lobe of bovine lactoferrin (prepared by proteolysis), the N-lobe of human lactoferrin (prepared by recombinant technology), and synthetic bovine lactoferricin peptides (Cornish et al. 2006). The N-lobes of both human and bovine lactoferrin and the C-lobe of bovine lactoferrin all showed osteogenic activity as measured by proliferation of primary rat osteoblasts, but the magnitude of response was less than for the full length molecule (Fig 6A). Interestingly, the bovine C-lobe appeared to have a stronger effect on proliferation than the bovine N-lobe. The bovine lactoferricin peptides (17-31 & 20-30) were both mildly osteogenic (Fig 6B). The N-lobe of human lactoferrin decreased osteoclastogenesis in a dose-dependent manner with an

activity that appeared to be equivalent, on a molar basis, to that of intact recombinant human lactoferrin. In contrast, the C-lobe of bovine lactoferrin has only a weak effect in this assay. The ability of the various lactoferrin lobes and fragments to influence both osteoblast proliferation and osteoclast development suggests that several sites on the lactoferrin molecule might be involved in receptor recognition, binding and stabilisation, or alternatively, more than one receptor might be involved. On a molar basis, the activity of intact lactoferrin on bone cell proliferation was at least 10-fold greater than that of the part molecules, which suggests that a global structure is required for optimal activity. In contrast, the equivalent osteoclastogenic activities of the recombinant human lactoferrin and its N-lobe suggest that this activity might be largely located in the N-lobe. However, further structure/function studies are warranted, as from a therapeutic perspective small active synthetic peptides might present a more attractive option for drug development than the intact lactoferrin molecule.

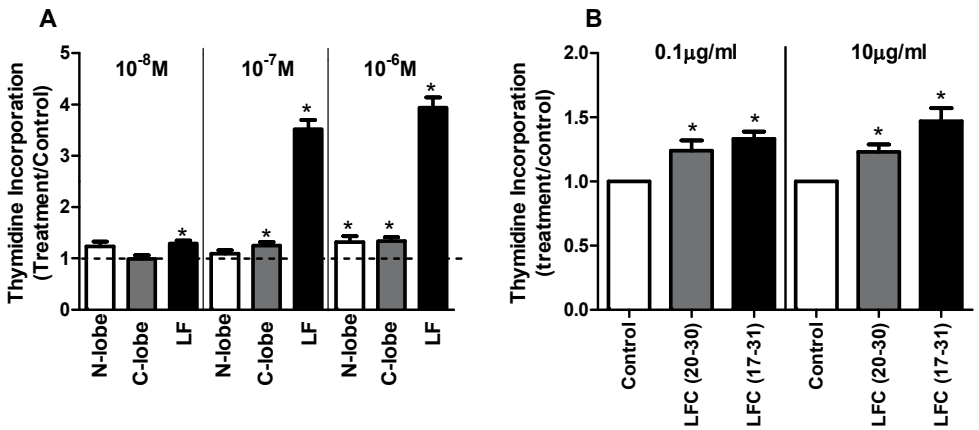


Fig. 6. Thymidine incorporation in primary rat osteoblasts treated with lactoferrin fragments. LF; intact lactoferrin, LFC; lactoferricin.

3.4 Lactoferrin's activity in bone *in vivo*

A number of recently published studies tested the potential use of lactoferrin for protection against bone loss. The effect of dietary supplementation of lactoferrin on bone was measured using ovariectomized (OVX) rodents as a model for post menopausal bone loss (Blais et al. 2009; Guo et al. 2009; Malet et al. 2011). C3H mice that were either OVX or sham operated, received a control diet or the same diet supplemented with different concentrations of bovine lactoferrin for 27 weeks. Lactoferrin supplementation improved bone mineral density and bone strength, measured as femoral failure load, in a dose-dependent manner (Blais et al. 2009). A study in OVX rats produced similar results. Lactoferrin orally administered to OVX rats for 3 months protected them against the OVX-induced reduction of bone volume and bone mineral density and increased the parameters of mechanical strength. Measurements of biochemical markers of bone remodelling indicated greater bone formation and reduced bone resorption occurred in rats treated with lactoferrin (Guo et al. 2009). Yamano et al. (Yamano et al. 2010) studied the potential use of lactoferrin for the prevention of alveolar bone destruction associated with periodontitis in an LPS-induced periodontitis rat model. Lactoferrin or liposomal-lactoferrin, which

improved the robustness of bovine lactoferrin to digestive enzymes, were added to the drinking water for 7 days. The study showed that bone resorption stimulated by LPS through activation of TNF-α production and modulation of RANKL/OPG balance in osteoblasts was inhibited by the orally administered lactoferrin. The researchers suggest that liposomal-lactoferrin could be a potent therapeutic and preventive agent for attenuating alveolar bone destruction in periodontitis patients.
In a small clinical study, 38 healthy postmenopausal women were randomized to receive placebo or a ribonuclease-enriched lactoferrin dietary supplement (Bharadwaj et al. 2009). In the lactoferrin-treated group there was a decrease in the bone resorption markers urine deoxypyridinoline (Dpd) crosslinks and serum N-telopeptides and an increase in the bone formation markers bone-specific alkaline phosphatase and osteocalcin, but the results are difficult to interpret due to differences in the levels of markers between the two groups before treatment.

3.5 The expression of lactoferrin in bone and cartilage

Investigations of the expression of lactoferrin in normal fetal and adult bone and cartilage by immunohistochemistry determined that fetal chondroblasts and osteoblasts are positive for lactoferrin immunoreactivity, whereas the corresponding adult cells are negative (Antonio et al. 2010; Ieni et al. 2009a; 2009b; 2011). Bone and cartilaginous specimens from fetuses at 8-34 weeks of gestation were studied. At the eighth gestational week, lactoferrin immunoreactivity was mainly present in the mesenchymal cells forming the periosteum and in chondroblasts; and a lactoferrin signal was also present in immature osteocytes and osteoblasts up to the 18th gestation week. The lactoferrin immunoreactivity decreased considerably by the 24th week, with no expression found in any bone area after the 30th week or in any samples from adult bone (Antonio et al. 2010; Ieni et al. 2011). The expression of lactoferrin in bone and cartilaginous tissue between 8 and 24 weeks of gestation suggests a possible role for lactoferrin as a bone growth regulator in the early phases of the human endochondral ossification.
The expression of lactoferrin was also studied by immunohistochemistry in a large number of tumors of bone and cartilage (Ieni et al. 2009a; 2009b; 2011). About half of all cases of osteocartilagineous tumors were positive, with lactoferrin expression in all giant cell tumors tested, all chondroblastomas, chondromyxoid fibromas and most osteoid osteomas. No lactoferrin immunoexpression was detected in osteosarcomas, chondrosarcomas, ossifying fibromas, osteochondroma and enchondromas. It is possible that lactoferrin expression reflects a less mature phenotype of these tumors, as lactoferrin is absent from normal adult bone and cartilage tissues.

3.6 Lactoferrin as a therapeutic agent

3.6.1 Local delivery

There is much interest in the potential use of lactoferrin as a factor that can act locally in topical applications for regenerative bone therapies and bone tissue engineering. Various biomaterials and biomedical devices have been used to improve delivery and enable sustained release of lactoferrin at the requisite site. Bovine lactoferrin incorporated into a type 1 collagen membrane promoted bone-like tissue formation by MG63 cells which were plated over the membrane (Takayama & Mizumachi 2009) and implantation of biodegradable gelatin hydrogels incorporating lactoferrin into a skull bone defect of rats

resulted in significantly stronger bone regeneration at the defect than was observed in either lactoferrin-free- or low-lactoferrin-treated rats (Takaoka et al. 2011). It was concluded that the sustained release from the gelatin hydrogels enabled lactoferrin to enhance the *in vivo* activity of bone regeneration. A titanium bone plate carrying lactoferrin for treatment of metaphyseal fracture has been patented, primarily as an implant for antibiosis, but such a device could conceivably be used for the promotion of bone repair (Fei et al. 2008). In the same context, a recent technology has been described whereby lactoferrin was coated in thin films onto inert substrates such as silica and biocompatibility assessed for use in applications such as implants (Constantinescu et al. 2009).

3.6.2 Oral delivery

The potential use of lactoferrin as a food supplement that promotes bone health requires experimental evidence showing that it is active when administered orally. Most ingested proteins are degraded into oligopeptides and amino acids in the small intestine and then absorbed as nutrients. The digestion of lactoferrin was studied in adult mice and rats (Fischer et al. 2007; Kuwata et al. 2001). In mice, immunoreactive lactoferrin, measured by ELISA one hour following intragastric intubation of a single dose, was present at the highest concentrations in the stomach, and in lower concentrations in all segments of the intestine: proximal intestine, distal intestine, caecum and large bowel (Fischer et al. 2007). Oral administration of ^{125}I-labelled lactoferrin in adult rats, followed by detection of multiple forms of degraded lactoferrin by surface-enhanced laser desorption/ionization (SELDI) affinity mass spectrometry showed that the bioactive fragment lactoferricin (17-42) could survive proteolytic degradation in the small intestine (Kuwata et al. 2001).

Transport of intact lactoferrin from the gut lumen to the circulation has been shown in infants (Hutchens et al. 1989, 1991; Knapp & Hutchens 1994) young calves (Talukder et al. 2002, 2003) and piglets (Harada et al. 1999) suggesting that as the selective transport from the gut is not yet fully developed macromolecules can cross into the circulation. In addition, a number of recent studies demonstrated transport of intact lactoferrin in adult animals and in humans. Fischer et al. (Fischer et al. 2007) found that 10 minutes after the administration of 1mg lactoferrin to adult mice through intragastric intubation, the intact molecule could be detected in the peripheral blood as well as in the liver, kidneys, gall bladder, spleen and brain. Transport of lactoferrin into the circulation has also been shown in groups of Ovx mice that were fed different concentrations of bovine lactoferrin (1-20 g/kg) for 27 weeks. Blood concentrations of immunoreactive lactoferrin of mice that received the bovine lactoferrin-supplemented diets were significantly increased compared to controls and were correlated to the bovine lactoferrin concentration in the diet (Blais et al. 2009).

3.6.3 Functional foods

An important consideration for the use of food systems as vehicles for bioactive delivery is that the bioactive remains active throughout manufacture and shelf-life of the product. Recent work has shown that bovine lactoferrin dosed into stirred yoghurt remained structurally intact and retained its osteogenic activity on primary bone-forming cells up to 21 days after storage of the yoghurt at 4° C (Palmano et al. 2011). In many respects yoghurt is the ideal functional food matrix for bone as it is calcium rich and a popular consumer product.

3.6.4 Lactoferrin preparations with potential use for bone applications

Bovine and human lactoferrin constitute the most studied of the lactoferrins. In general and in spite of some structural differences, bovine and human lactoferrin including recombinant forms appear to have comparable bioactivities. However, some differences between bovine lactoferrin and human lactoferrin have been noted with respect to intestinal receptor recognition (Kawakami & Lonnerdal 1991) and it cannot be assumed that activities are always interchangeable

The use of lactoferrin as a therapeutic agent requires not only proof of efficacy at the clinical level, but assured safety, consistent quality of supply and appropriate delivery mechanisms. Most studies on the effects of lactoferrin on bone, including clinical trials, have been performed using bovine lactoferrin. Bovine lactoferrin from milk has been available as a commercial isolate for many years (Tomita et al. 2009). It has a 'Generally Recognized As Safe' (GRAS) status from the United States Food and Drug Administration (FDA) and now has widespread acceptance for oral use in humans. Indeed, it has been available for a number of years in Japan and other countries as a dietary supplement and as a functional ingredient in foods such as yoghurt and fortified infant formulae (Wakabayashi & Tasaki 2006). Bovine lactoferrin has been administered orally at doses of 3g/day for one year in a cancer clinical trial, with some positive outcomes and no apparent adverse effects (Tomita et al. 2009).

Equally, recombinant human lactoferrin can be considered for oral application although to date there have been no oral efficacy clinical trials targeted specifically at bone. High expression levels can be achieved in rice (Nandi et al. 2005) and transgenic animals (van Berkel et al. 2002) and recombinant human lactoferrin expressed in both baby kidney hamster cells and rice has been shown to have comparable activity to human lactoferrin and bovine lactoferrin in stimulating proliferation of primary rat osteoblasts (Cornish et al. 2004; Huang et al. 2008). Rice recombinant human lactoferrin was shown to have no toxicity in rats when administered up to 1000 mg/kg body weight/day for 28 days (Bethell et al. 2008a, 2008b) and did not elicit an allergic response in plant-glycan sensitive humans in a limited clinical study (Mari et al. 2008). Moreover it was shown to have beneficial effects as an oral agent in a clinical trial targeted at reduction of diarrhoea in Peruvian children (Zavaleta et al. 2007). No adverse events were reported.

Another potential candidate for bone interventions is Talactoferrin alpha, a proprietary recombinant human lactoferrin expressed in the fungus *Aspergillus awamori* and produced at industrial scale by Aggenix AG (Sanchez et al. 2010). Talactoferrin is currently being evaluated for the oral treatment of several cancer types and Fast Track designation has been granted to Agennix by the FDA for treatment of non-small cell lung cancer (NSCLC) and first-line treatment of renal carcinoma in combination with sunitinib. Placebo-controlled Phase II clinical trials have been successfully completed for NSCLC (Jonasch et al. 2008) and at time of writing two Phase III trials evaluating Talactoferrin in NSCLC patients are ongoing. Talactoferrin appears to have no toxicity, is well tolerated and also appears to be safe for topical applications. It has shown efficacy in the local treatment of diabetic ulcers (Engelmayer et al. 2008) for which it also has Fast Track FDA approval (Sanchez et al. 2010).

4. Conclusion

The positive effects of lactoferrin in bone have been demonstrated *in vitro*; where lactoferrin induces osteoblast proliferation, survival and differentiation and inhibits osteoclast formation, and *in vivo*; where lactoferrin given as a dietary supplement to rat and mice

protects against bone loss associated with oestrogen deficiency. The molecular pathways activated by lactoferrin in bone cells are only partially understood, and it appears that a combination of direct and indirect physiological mechanisms is producing the overall anabolic effect of lactoferrin in bone. Pharmaceutical or nutriceutical use of lactoferrin would require the development of a preparation with assured safety and consistent quality of supply. A better understanding of lactoferrin's mechanism of action in bone would allow for the design of compounds that can mimic its anabolic bone activity, and would be useful in pathological states of reduced bone quality in either systemic or local applications.

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How Dentistry Can Help Fight Osteoporosis

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1. Introduction

The late 20th century has brought to many patients a promising horizon: the lust of aging. Implicit in this "present" in longevity, however, is the known adverse changes in body tissues. The consequences of aging often involve the risk of osteoporosis, leading to an impaired quality of life of the elderly patients. Morphological and functional oral sequelae of aging are well documented in dental literature, but not those resulting from osteoporosis. Many authors have cited the possible correlation between age, systemic osteoporosis, periodontal disease, tooth loss, and changes in quantity and quality of bone of the maxilla and mandible. The restoration of occlusion for partially and totally edentulous patients often requires adequate bone therapy. Consequently, the frequent use of implant-supported prostheses for elderly patients who are routinely or potentially osteoporotic demand a better understanding of the relationship between osteoporosis, the stomatognathic system and muscle insertions. The jaw is constantly subjected to masticatory forces, movements during speech, breathing, and swallowing and is therefore an adequate structure for studying bone quality.

Panoramic radiography produces an image that displays both maxilla and mandible, the teeth, their supporting structures, and other important structures such as the nasal cavity, maxillary sinuses, temporomandibular joint, styloid process, and often to the bone hyoid. Although dentists routinely focus interest on the teeth and alveolar ridges when examining some panoramic radiograph, they should also be able to consider major changes in other structures that appear in the image (WHITE et al, 2004; FARMAN et al, 1993; WATANABE et al, 2004).

"The Selection of Patients for X-Ray Examination", US Food And Drug Administration Center for Devices and Radiological Health (FDA/CDRH), or "GUIDELINES FOR PRESCRIBING DENTAL RADIOGRAPHS", GPR, was first published in 1987 using the American population (USA), considering the total exposure to ionizing radiation arising from any source. In 2004, the GPR has been updated and published once more after hard work of a board of experts from the "American Dental Association and Food Drugs Administration" (ADA, 2004). The actual GPR expanded the use of panoramic radiography, proposing this technique as the first alternative for supplementary examination for dental diagnosis, recognizing the great technological advancement and improvement of the equipment for panoramic radiographs.

While the panoramic radiograph should not be prescribed primarily for the detection of the systemic conditions affecting the maxillomandibular region, we must recognize that in the

routine of health professionals, dentists, is used for initial assessment of dento-alveolar conditions, and it would be important that these dentists recognize certain conditions in these radiographic images that indicate the presence of systemic diseases. It should be understood that "systemic disease" means conditions that are spread inside the body, rather than localized primarily or only in tissues of the oral cavity. Below we will mention and describe some conditions on the images on panoramic radiographs that suggest significant disease extent, enough to affect quality of life and longevity of patients. A major disease that afflicts mainly men and women in old age is osteoporosis.

"Osteo" is Latin for bone. "Pores" means "full of pores or holes." Thus, osteoporosis means "bones that are full of holes." The bone mass reflects the balance between formation by osteoblasts and resorption by osteoclasts. Around the third decade of life the peak bone mass is reached, and then begins a slow process more continuous bone loss progresses with age. Osteoporosis is a multifactorial metabolic bone disease characterized by low bone mineral density (BMD), the deterioration of the microarchitecture of cancellous or trabecular bone, and changes in the physical properties of bone, leading to increased bone fragility with a consequent increase in fracture risk mainly of bones like the femur, forearm and spine (Fig.1). In the case of the oral cavity, the biggest consequence of this damage is the resorption of the alveolar ridge and possibly teeth loss as well as providing poor quality bone for the installation of oral implants. Nevertheless, it may also lead to mandibular fracture.

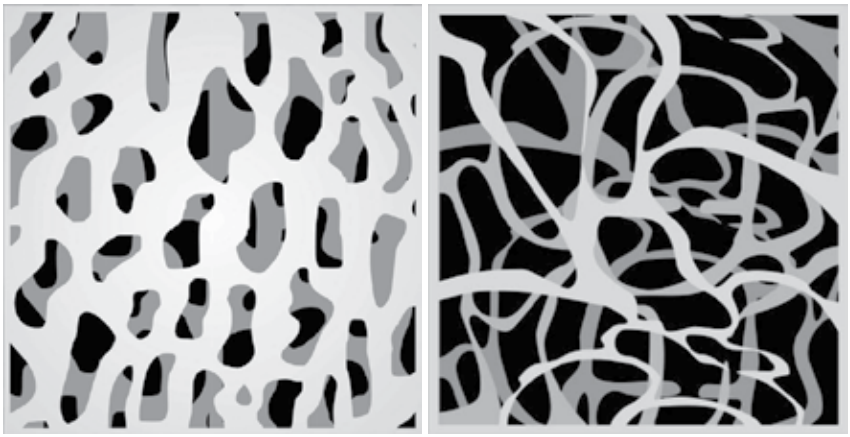


Fig. 1. Schematically drawing of the normal bone trabecular tissue, showing a trabecular net of thick and linked. Already to the right we see sharpening trabecular tissue and a net with bigger medullar spaces.

Osteoporosis is the process of quantitative loss of bone density per unit volume, with maintenance and reduction of the qualitative properties of mineralized bone. This change is responsible for the imbalance of the mechanics of the skeleton, increased number of fractures, notably in the spine, femoral neck and distal segment of the radio. The most common structural changes are the reduction of trabecular bone in size and number, and the thinning of the cortical region, with greater involvement of trabecular structure. This can also be seen in the maxillomandibular region, most obviously in the jaw, with decreased cortical thinning and inferior mandibular body.

Osteoporosis in postmenopausal women in the United States constitutes a public health problem because it affects 25 million women, with an annual average of 1.3 million fractures

and estimated cost of seven to ten billion dollars. In Brazil, although the fragility of our statistics do not allow further information, we can say that the problem worsens each year, mostly by increasing the relative population of menopausal women and increased life expectancy of this group. So, osteoporosis is a public health problem, affecting millions of people worldwide. Like any other disease, osteoporosis affects the self-esteem of the patient and leads to complications in the family routine, generating costs for the public health system in the patient treatment. These concepts resonate across the world, and especially in Brazil, a developing country with explicit socio-economic-cultural deficit.

Asymptomatic progression of osteoporosis, in conjunction with the possibility of catastrophic disability, this disorder is the biggest public health priority in many countries. Osteoporosis can progress asymptomatically until a bone fracture or a dental loss. One in two women and one in eight men over age 50 will develop osteoporosis. If the disease occurs, 15% and 20% of women will need special care for long periods due to loss of the ability to manage basic activities at home. Half of the persons who suffered a hip fracture lose their ability to live independently, and around 20% of this persons will die within a year as a result of the fracture (COSMAN & LINDSAY, 2004), (Fig.2).

Osteoporosis is not only a woman's disease. Not as many men have it as women do—maybe because most men have more bone density. As they age, men lose bone density slowly than women. However, men also need to be aware of the risks of osteoporosis and men have more fractured femurs than women.

Fig. 2. Some data alarming epidemiologists on osteoporosis in the world.

In Brazil, the prevalence of osteoporosis is poorly understood (SS-HSPE, 1995); however, in 2001 the osteoporosis clinic at UNIFESP measured the use of public resources and the annual cost for patients with postmenopausal osteoporosis. The average annual cost for the patient was approximately $442.00 per patient. However, some authors[9] assessed the direct cost during hospitalization for an osteoporotic hip fracture in the private health system, such as health insurance coverage, and the authors concluded that the cost was approximately $ 6.900/patient (KOWALSKI et al, 2001). The study, "Osteoporosis - 2000 of Brazil", developed by 300 medical experts, estimated that less than one-third of Brazilians who have osteoporosis diagnosis of disease, and that only 20% of those are receiving treatment (ARAUJO et al, 2006; MARQUES NETO & LEDERMAN, 1995).

2. Singular characteristics in geographical location

2.1 Emergent country

Food and / or nutrition are some of the main factors that affect bone quality as a whole, and significantly influence the osteoporosis disease in the world.

As to the geographical aspect, we should mention that Brazil, for example is between the equator and temperate zone just below the Tropic of Capricorn, which clearly favors the protection against the deleterious effects of osteoporosis, because fortunately, as a tropical country, received the largest part of the year the sunlight that is essential to activate vitamin D. Vitamin D (or calciferol) is a vitamin that promotes the absorption of calcium (after exposure to sunlight), essential for normal development of bones and teeth; it also acts as newly discovered immune system, heart, brain and in insulin secretion by the pancreas. This has primary function in the absorption of calcium in the body.

Among the environmental factors involved in osteoporosis, nutrition, particularly with respect to consumption of protein, dairy products and vegetables, has been named as a participant in the formation of bone mass (ANDERSSON 1999; RIZZOLI & BONJOUR, 1999; ROUSSEAU, 1997). The calcium and vitamin D during childhood seem to play an important role in the health of bones (WARDLAW, 1993). Retrospective studies in adults suggest that calcium intake in the first phase of development are associated with the risk of developing osteoporosis and fractures during adulthood (STALLINGS, 1997; VON MULHEN et al, 1999). However, we must consider the sensitivity of this nutrient absorption varies depending on the genetic constitution of the individual (MAY et al, 1994).

This privileged geographic location facilitates the cultivation of a variety of foods (from temperate and tropical climates). Fishing, and therefore the habit of eating fish is extremely encouraged by the fact that our country has an extensive Atlantic coast, more than 8000 km, and thus favors the fishery, which is quite diversified. Including food fish in the daily diet can greatly contribute to bone quality, mainly due to calcium that food provides. Fish is a major source of calcium along with other foods such as milk, yogurt, vegetables, nuts and cereals (Table 1).

This geographical location, yet is related to the sociological aspect, therefore, should remember the mixture among Indians, Portuguese and African blacks and among immigrants who came to Brazil from the nineteenth century, attracted by the opening of the immigration movement. Italian families, German, Portuguese, Spanish, Polish, Japanese and Arabs introduced their eating habits in the regions where they settled. These people, mainly Asians and Caucasians, are the main osteoporosis risk groups, owing much to the genetic characteristics. Even with respect to indigenous peoples, one of the most accepted theories of his presence in the Americas, is the migration of peoples from Asia across the Bering Strait (Bering Strait is a strait between Cape Dezhnev, the easternmost point of mainland Asian and Cape Prince of Wales, the westernmost of the American continent). During the last glacial era, with the recession of ocean water, the area of the Straits has become a natural bridge between Asia and the Americas, now called the Bering Land Bridge , where they could have reached America the people who first colonized). So you can see how confusing it is analyzing the DXA scans, which has standard tables to compare the bone mass values determined by tests of X-ray absorption, mainly due to the intense miscegenation of the population in Brazil (Fig. 3).

But we can go further in this confused analysis in our country, commenting on the various geographic regions of Brazil and food characteristics.

The native indians of the Region North had as basic food the "mandioca". The fish also represent an important parcel of the feeding, being the most consumed "tambaqui", "traíra", "piranha", fished, sardine of river, "tucunaré", "pacu" and "pirarucu" (called "cod the Amazônia"). All rich ones in sodium, potassium and calcium.

Beyond the influences aboriginal, Portuguese and black, the Northeast region received contributions from dutches, Frenchmen and English that had invaded the territory had dominated and it during a time. The result is a rich and varied culinary, that came to characterize the food of the region. With exception of the blacks, all the other peoples are group of risk osteoporosis.

The region Center-West, possesss innumerable families of colonists of the states of the South. These colonists had wide experience in cattle agriculture and modern. Of this form, its culinary was conditional to the resources of the environment, especially of fishes and the hunting, as: "pacu", "piranha", "golden", "painted", "anta", "cotia", "paca", "capivara", deer and alligator. Thus, this feeding is positive in relation osteoporosis.

In the region Southeastern they are the states richest of the country. Its food received diverse influences, that follow the history of the settling: of the indians; Jesuits; tamers (bandeirantes); Italian immigrants, of the Spaniard and Arabs in *Rio de Janeiro*, and of the Germans and Italians. Here also influences we see it sociological of these European, caucasianos peoples, groups of osteoporosis risk.

The South region was the one that received greater influence from immigrants. This because the tempering climate of the region was more similar to the European climate, facilitating the adaptation of the Italians, Germans, Poles and ucranianos, that if had established preferential in agricultural activities.

Table 1. Brazilian geographic regions and the main alimentary characteristics. Demographic census. Brazil, 2000.

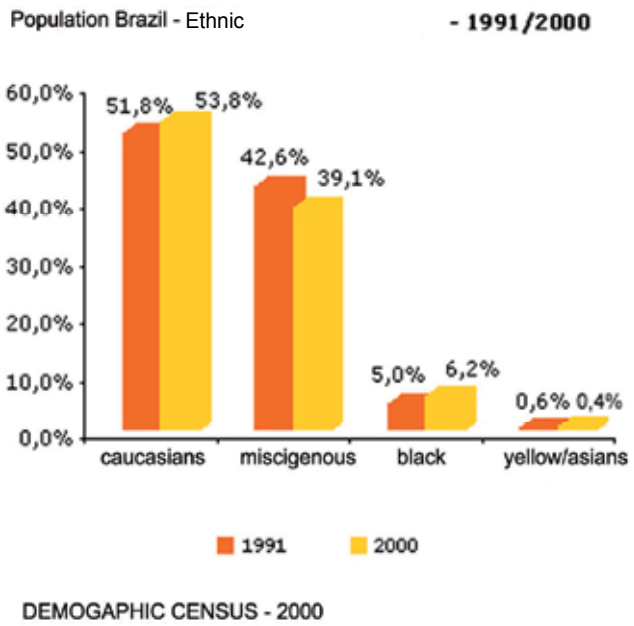


Fig. 3. Composition of the Brazilian population for race, 1991/2000 (IBGE-Brazilian Institute of the Geography and Statistic)

Caffeine, for example, is a food consumed by millions of Brazilians and their effect has been studied on bone quality. In 2002, Heaney conducted a literature review and concluded that "there is no evidence that caffeine has the harmful effect on bone status or calcium deposition in individuals who eat the recommended amount of calcium per day, or more than 3 cups of coffee. In our studies we have observed that there is a significant increase in the circulating calcium in rats that ingested caffeine daily, causing obvious radiolucency of the mandibular bone, the tibia and femur.

There are at least 31 recent cross-sectional studies, case control and cohort studies (observational studies are where individuals are selected or classified) according to exposure status, being followed to evaluate the incidence of disease, combining caffeine intake and bone health involving many thousands of patients (PINTO NETO, 2002). Aspects of bone health measured include BMD, its changes, the rate of fracture and osteoporosis. These observational studies could demonstrate only associations and not relationships of cause and effect.

Although the reviewed evidence is contradictory, the weight of evidence does not support the idea that beverages containing caffeine adversely affect bone health. The reason for the contradictory results is unclear. Taking as an example study, the inverse association observed before adjustment for obscure aspects between intake of caffeinated beverages and bone mass, disappeared after adjusting for other risk factors (JOHANSSON et al, 1992). It would also be possible that the intake of caffeinated beverages is acting as a marker for a true causal factor. It is known that there is an inverse relationship between milk intake and consumption of beverages containing cafeína (BAUER et al, 1993). It is possible, therefore, that a low intake of milk instead of a high intake of caffeinated beverages is the true cause of ill health óssea (HALLSTON et al, 2006). In 2009, some authors (WAUGH et al, 2009) published a systematic review of checking the risk factors for low bone mass in healthy

women aged 40-60 years. They found that there was good evidence that low body weight and Postmenopausal (PM) status are risk factors for low bone mineral density, and also found that there is good evidence that ingestion of alcohol and caffeine, and reproductive history were not factors risk. The results of a recent study (VONDRACEK et al, 2009), however, contradict these results. Changes in the lifestyle and healthy habits to favor the bones, such as calcium and vitamin D nutrition, regular exercise, limiting consumption of caffeine and alcohol, and the fact that tobacco smoking is not essential to the management of risk of osteoporosis. (Figure 4)

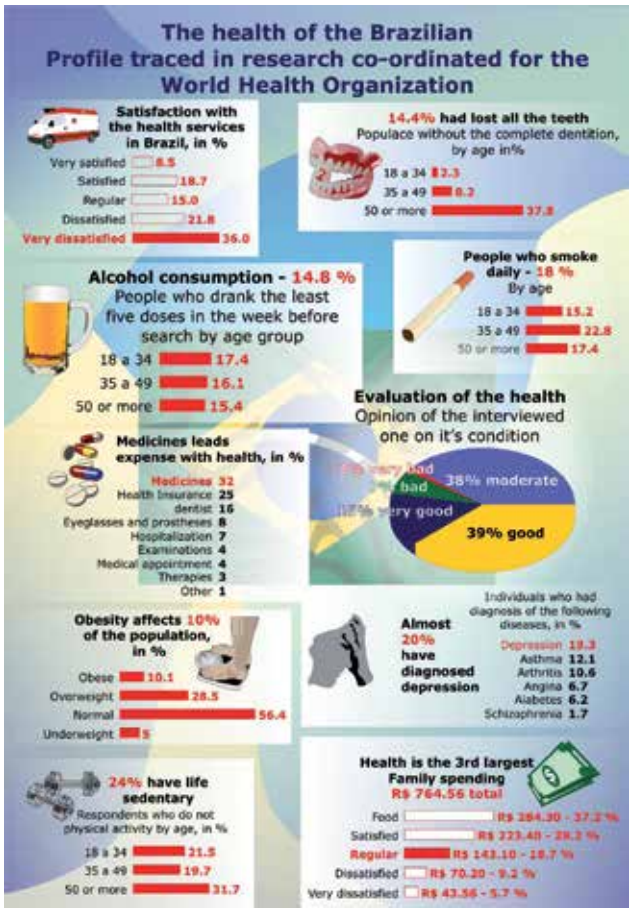


Fig. 4. Relative illustration to the health of the Brazilian (FIOCRUZ/WHO, 2008.)

Radiographic factors of osteoporosis in the skeleton include generalized osteopenia which is always more prominent in the column, cortical thinning, and accentuation of primary trabeculation and loss of the secondary trabeculation. Osteoporosis can be linked to pain, especially in the lower back. It can also result in pathological fracture, loss of stature, and severe kyphosis.

Radiological factors of osteoporosis in the mandible include relative radiolucency of the jaws and jaw and defining the reduced cortical, and erosions (Fig. 5 and 6). At the early stages of the disease is possible to find a sharp contrast from the oblique line of mandible,

mainly due to loss of trabecular bone mass, which leaves the body more mandibular radiolucent, accentuating the contrast effect in relation to the oblique line. The precision with which the panoramic radiographs can be used to assess the likelihood of a person having osteoporosis is still debated, with evidence being divided, polarized rather than for or against.

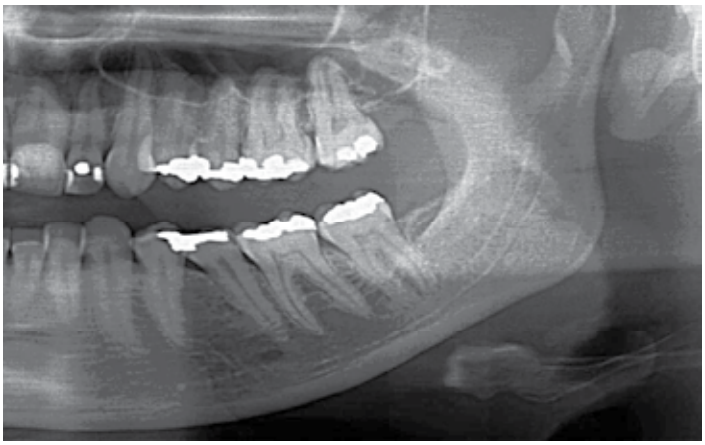


Fig. 5. Interest Region . Normal Mandibular inferior cortex – Cropped panoramics images

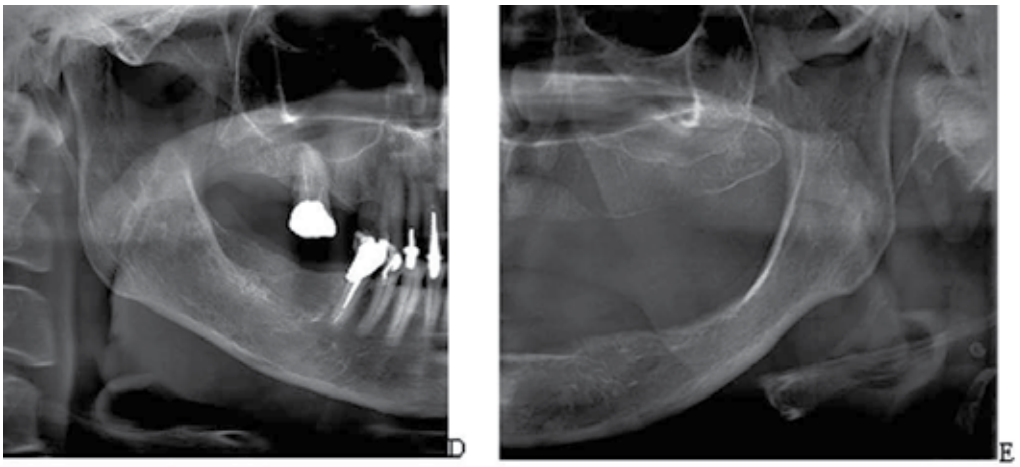


Fig. 6. Osteoporosis – Cropped panoramics images shows a relative radiolucency of both jaws with reduced definition and mandibular inferior cortex moderately eroded, evidence of lacunar resorption (right-D) or cortex severely eroded (left-E),

3. Population in developing countries will be the most affected

Currently, according to WHO, the majority of hip fractures due to osteoporosis happens in the countries of Europe and North America. This projection is based on the fact that the demographic changes that must occur within the next 50 years will significantly increase the number of elderly in Asia, Africa and South America. According to the Brazilian

Institute of Geography and Statistics (IBGE), the group 30 to 59 rose from 25% of the population (in 1940) to 32.9% (1998), and is expected to reach 40.2% in 2020. The elderly above 60 years, amounted to 12.4 million people in 1998 and may be 25 million over the next 21 years. We must emphasize that life expectancy measured in the last IBGE Sense is 73 years on average.
In Europe, every 30 seconds someone suffers a fracture due to osteoporosis. It is estimated that in 2050, Latin America and Asia, the incidence of hip fractures due to bone disease should be for one in two cases. In Asia, according to medical records, is considered more dramatic the expected increase in cases of hip fractures in the coming decades. Other studies show that in the Middle East will triple the number of hip fractures caused by disease in the next 20 years. Cosman & Lindsay, 2004.

4. Bone loss: Clinical implication

The major consequence of bone loss (PO) in our aging society is fracture. In the oral cavity could be considered tooth loss, affecting 26 million people in Brazil, according to the IBGE. Oral health of Brazilian social inequality is indicative of the country. According to the Brazilian part of the World Health Survey, released by Fiocruz (Oswaldo Cruz Foundation) and held last year at the WHO (World Health Organization), 14.4% of Americans have lost all their teeth (SZWARCWALD & VIACAVA, 2005).
Taking into account that the IBGE estimates 179 million in the current population in Brazil, that means about 26 million no longer have any natural teeth. The comparison between the social classes shows that, among the poorest, this percentage reaches 17.5%, and among the richest, is 5.9%. Fiocruz made the separation between classes by the number of consumer goods (televisions, refrigerators, etc.). The worst situation was found among women over 50 in poor families: 55.9%. That is, postmenopausal women are main group at risk for osteoporosis. The researchers compared risk factors for health. The data show that 10.1% of the population can be considered obese, according to the WHO standards, and that 28.5% of Americans are overweight. The percentage of underweight people is 5%. These people probably have very brittle bones, and thus risk of systemic osteoporosis. The rate of those who said they had drunk at least five servings of alcoholic beverages in the previous week is about 14.8%, a percentage that is higher in the younger age group (18-34 years), which reaches 17.4%. Daily smokers represent 18.1%, and the habit is lower among those between 18 and 34 -15.2%. It is a fact that excessive alcohol and smoking affects bone mass and quality of oral health. The poll found that 24% of the population is sedentary. This is a major factor influencing the quality of the bone skeleton.

4.1 Measurements based on radiographs

Many physicians believe that an X-ray may also be appropriate after experiencing a loss in height or a change in posture, or some alteration in the skeleton. This is an old concept that still exists. One should remember that this is a concept of more than 50 years, when radiology still worked with slow films, with technology of grains in emulsions, ecrans that emitted blue light, 16 times more radiation and chemical processing. Nowadays, radiology works with little radiation, X-ray equipments are more accurate, conventional films have the technology of tabular grains, ecrans that emit green light (more sensitive), and still digital

images, with ample possibility in computers that increase the diagnostic capacity. This has led professionals to change their thoughts in relation to the older concept.

4.2 X-ray absorptiometry: Dental radiography

X-ray absorptiometry is a technique widely utilized for measuring bone mineral density (BMD). The low correlation among densitometric results obtained for distinct bone sites valuation imposes the development of a technique for accurate mineral density assessment on maxillary and mandibular bones specifically, adequate for dentistry procedures. For maxillary and mandibular bone mineral density measurements by single energy x-ray computed absorptiometry, periapical intraoral radiographs can be taken using an aluminum densitometric scale. Watanabe et al, 2008 mesured the density values on 55 adult patients that were arranged in four population groups, with distinction among maxillary and mandibular bone and patient gender. The measurements were statistically evaluated, resulting in the determination of the population reference data based on the average density and respective standard deviation for each group. Assuming one standard deviation as the confidence interval, the lower threshold for normality corresponds to bone mineral density of 2.00 mm for women maxillary bone, 3.28 mm for women mandibular bone, 3.88 mm for men maxillary bone and 5.45 mm for men mandibular bone. These thresholds were implemented on Cromox ® DOMM 3.2.2 system, for normal and osteopenia distinct diagnosis, associated to low effective radiation dose on a technique with more comprehensive use in the population (WATANABE et al, 2008). (Figure 7 and 8)

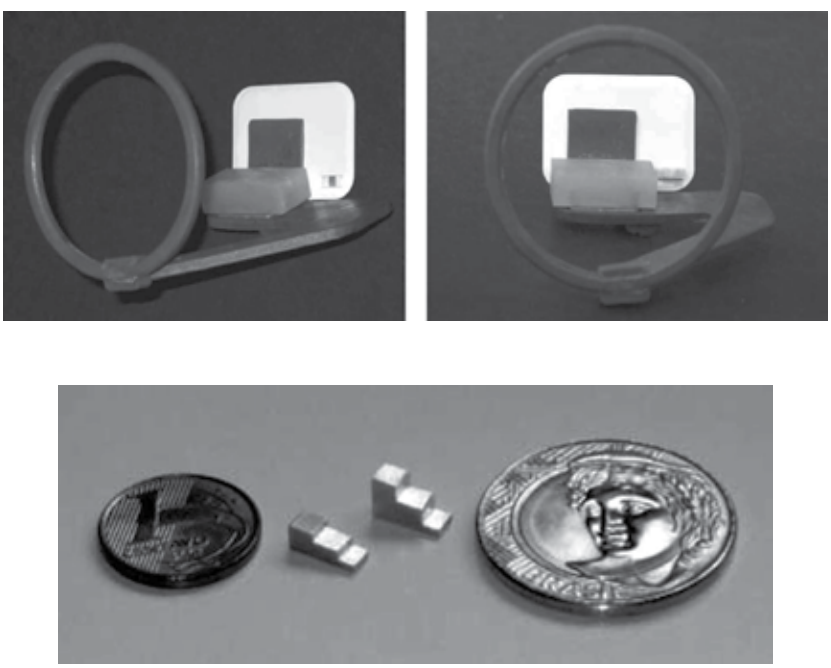


Fig. 7. Prototypes III and IV of the densitometry scale (coins are used as a size reference).

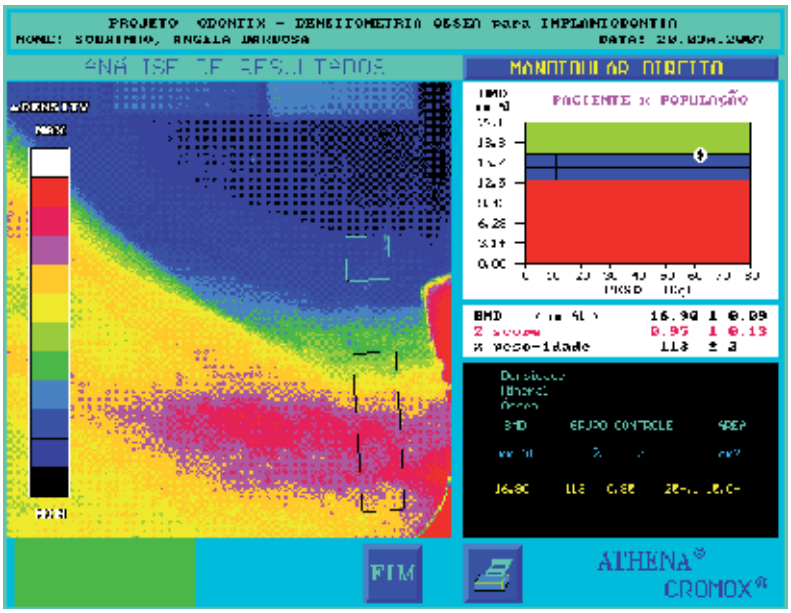


Fig. 8. Densitometric exam of the mandible in the Software Cromox

5. Radiographic signs of osteoporosis in dental radiography

5.1 Panoramic and oral radiography

Dentists are in a potentially valuable position for patient screening for signs of osteoporosis; significant portion of the population visits their dentist annually and dental radiographs are prescribed for many. In the last four decades, numerous researchers have reported that osteoporosis can be diagnosed through oral radiographs; panoramic radiography is widely used for routine dental examinations and it would be very useful to determine if radiographic changes in the mandible could show skeletal osteopenia and have an important role in detection of osteoporosis. Thickness of the inferior border of the mandible below the mental foramen has often been measured as the panoramic mandibular index (PMI) either directly or as a ratio of the thickness to the distance of the mental foramen from the inferior border. (Fig. 5)

The use of panoramic radiography is common in a dental setting and is also advocated by the International Guide to Prescription Radiographs[4]. Digital radiographs are an increasingly popular option in the clinic. Such images are composed of pixels with a specific numerical value for each one. Two important methods of evaluating the pixels in these images are Fractal dimension (FD) and Pixel Intensity (PI) analyses. FD is expressed numerically and consists in describing complex shapes and structural patterns in the bone. PI is a grayscale measure, ranging from zero (black) to 256 (white) in a 8-bit digital image (VON MULHEN et al, 1999). Because the panoramic radiograph is an exam more common and affordable than DXA, its application in the early detection of low bone mass would bring significant benefits for the treatment of osteoporosis (VON WOWERN, 1986).

The cardinal radiographic signs of osteoporosis in the skeleton include osteopenia generalized thinning and the accentuation of corticosteroids in the bones, and the accentuation of the trabecular bone. The factors include spontaneous fracture, and

traumatic, especially the spine, wrist, hip or spine, invagination at the base of the skull and bones of grainy skull (VON WOWERN, 1986). The main radiographic signs of osteoporosis in the maxilla and mandible (Figure 2) include a generalized radiolucency on both the maxilla and mandible, as evidenced by defining the cortical or accentuation of the maxillary sinus, nasal cavity, oblique line, and others. Where you can see some cervical vertebrae in panoramic radiography, the appearance of the "frame" of the bones can also be observed. A morphometric analysis of bone in cross section (VON WOWERN, 1986) showed that the structure of the jaw bones and jaw in dentate elderly, is characterized by cortical porous, relatively thin, with demineralization of the bone endosteum, as in other skeletal bones, and these changes age-related cortical tend to be more common in women than in men. The bones of the jaw and jaw variations between individuals and regional structures and density of trabecular bone may mask the decrease in bone mass that is related with gender and age, as seen in other trabecular bones of the skeleton. The methods for evaluating these age-related changes in the maxilla and mandible were listed by Bras et al, 1982.

6. Panoramic radiography

The integrity of the bone microarchitecture is an important element of the bone quality and contributes for the mechanical abilities of the bone (FARMAN *et al*., 1993; TAGUCHI, 2004; BOUXSEIN, 2003; SEEMAN, 2003; WOWERN, 2001).

RESEARCHERS	THECNICS	Regions	Measures
Bras *et al*. (1982)	Panoramic Radiography	----------	Cortical width Ratio: width of the cortical one with in the distance of the inferior edge of mentual forame for the inferior edge of the jaw
Benson *et al.* (1991)	Panoramic Radiography	----------	
Klemetti *et al.* (1994)	Panoramic Radiography	----------	Basal Cortical, classification: C_1; C_2; C_3, endosteals edge.
Kribbs *et al.* (1992)	Intramural film with Aluminun penetrometer	Gonio, mentual forame, and mandibular molar region.	Bony density by microdensitometer.
Wowern (1993)	Dual-Foton absorciometria (DPA), mandible.	Basal of the mandibular molar region	Bone Mineral Content (BMC), g/cm^2.
Corten (1995) Horner *et al.* (1998)	X-rays Dual Emission (DXA)	Edentulus Mandible	BMC em g/cm^3, both sides .
Bassi *et al.*(1999) Klemetti *et al*.(1995) Lindth *et a*l.(1996) Taguchi *et al*.(1996)	CTQC Dual or Single Energy.	Mandible	Bone Mineral Density – cortical and trabecular bony, separately horizontal in mg/cm^3

Table 2. Original methods for evaluation of the changes of the bone of the jaw *in vivo*

6.1 Radio-morphometric indices (Table 2)

- Mentual Index (MI) (LEDGERTON et al, 1997, 1999): the cortical thickness in the mental region can be measured using a modified technique from the primarily described by Ledgerton *et al.* Initially, the mental foramen is identified and then a line is drawn perpendicularly from the top edge of the mental foramen to the bottom edge of the mandibular body, where another line was drawn to serve as reference to obtain the mandibular cortical bone thickness at a sharp angle (normal greater than or equal to 3.0 mm) (Fig. 6-7);
- Panoramic mandibular index (PMI) (BENSON et al, 1991; ANDRADE at al, 2009), (Fig. 6-7)

The measurements can be made in the panoramic radiographs in the mental foramen area, with the aid of the RADIOIMP software (RADIO MEMORY LTDA, version 2.0). When opening the image of the panoramic radiograph, the program requests the calibration of the same, where the following information should be inserted:

1. Type of equipment used: e.g. SuperVeraviewscope.
2. Image Resolution: 300 dpi.
3. Equipment Magnification: 30%

After the simultaneous alteration of the brightness and contrast tool of the images for better visualization of the mental foramen area, the measurements can be initiated, according to the technique proposed by Benson et al, 1991 based in the technique of the Wical & Swoope (1974). The first step is the identification and the tracing of the mental foramen unilaterally; then, a parallel line will be drawn down the long axis of the mandibular body and tangent to the inferior border of each side. Later, the mandibular cortex will be measured, through a line drawn perpendicularly from the first and afterwards the height of the mental foramen will be also measured, given by the distance of the inferior border of the foramen to the base of the mandible. All the measured lines presented a 90º degree format. For better identification of the measured area, the zoom tool will be also used to facilitate the the measurements, as the presented values were around 0,3 cm (Figure 9 and 10).

The thickness of the mandibular cortex is divided by the distance between the mental foremen and the inferior mandibular cortex to obtain the PMI (BENSON et al, 1991).

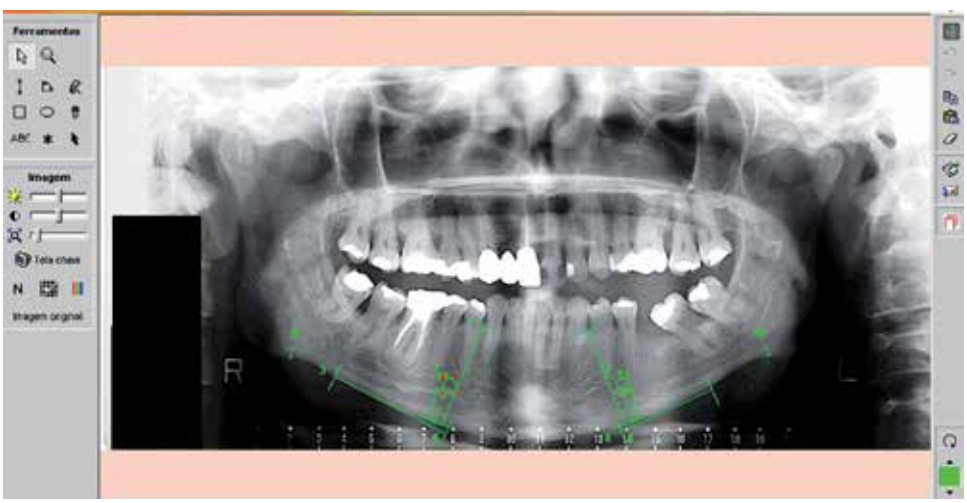


Fig. 9. Measurement of the Mentual and Antigoniac indices. Radioimp-RADIO MEMORY LTDA, version 2.0

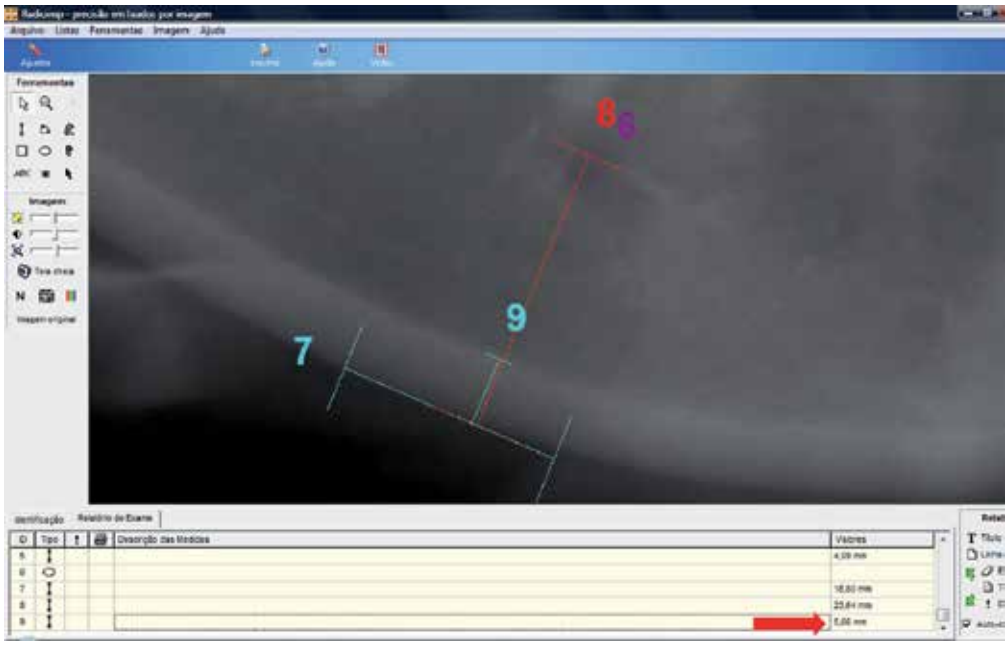


Fig. 10. Mentual index in detail.

Klemetti Classification

The mandibular cortical shape is classified into one of three groups according to the method of Klemetti et al, which considers qualitatively the endosteal margin of mandibular cortical (KLEMETTI et al, 1994): C1—the endosteal cortical margin is even and sharp on both sides, normal cortex (Figure 11); C2—the endosteal margin has semi-lunar defects (lacunar resorption) or endosteal cortical residues on one or both sides (Figure 11), mild to moderate cortex erosion; C3—the cortical layer forms heavy endosteal cortical residues and is clearly porous, severely eroded cortex (Figure 11).

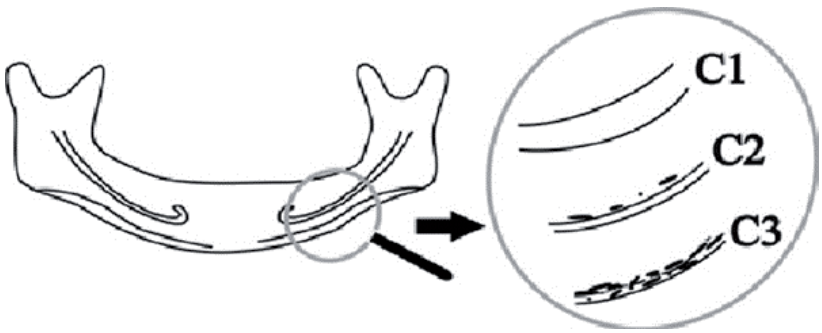


Fig. 11. Klemetti classification

- Antegoniac Index (AI) (DUTRA et al, 2005; MAHL et al, 2008) – mandibular cortical thickness measured on a line perpendicular to the mandibular cortex at the time that it crosses the tangent to the anterior edge of the industry (normal value greater than or equal to 3.2 mm);

- Goniac Index (IG) (DUTRA et al, 2005) – mandibular cortical thickness measured on the bisector of the angle between the tangent lines to the posterior border of the ramus and the mandibular base (normal greater than or equal to 1.0 mm). (Figure 12)

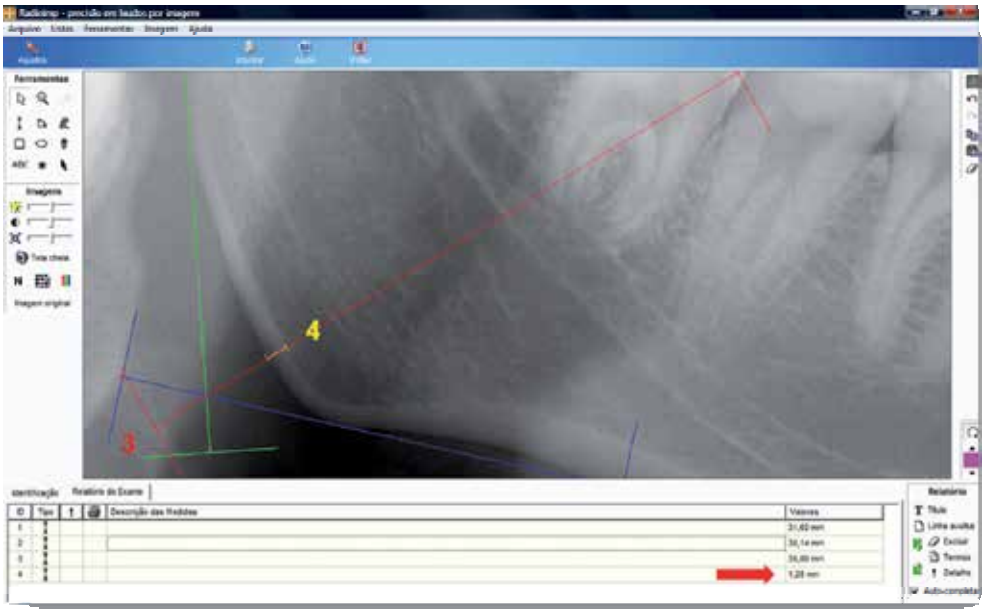


Fig. 12. Method of the measured Goniac Index (IG)

In the study of indices proposed in radio-morphometric mandible (TAGUCHI et al, 1995) it was found that the indices evaluated were reproducible; PMI and MI showed the highest sensitivity for detecting osteopenia / osteoporosis, but the specificity of the panoramic mandibular index was low, all the indices evaluated were able to identify low bone mass, however, only PMI and MI could differentiate patients with osteopenia / osteoporosis.

7. Evidence to support panoramic radiography for the diagnosis of osteoporosis

If persons at risk of osteoporosis can be screened using panoramic radiographs, screening of persons without subjective symptoms that are difficult to diagnose or persons without concern for osteoporosis, and instruction for only persons potentially having osteoporosis to undergo closer examination such as DXA or referral to a facility equipped with that apparatus allow early detection and early treatment of patients suffering from osteoporosis, and also reduce costs of the examination. Furthermore, the method used for this screening must be simple and usable even without having any special skills or requiring complicated operations.

The relationship between osteoporosis and oral signals was investigated to evaluate the possibility of using this as an indicator of osteoporosis. Some authors Taguchi et al 1995 studied 64 postmenopausal women aged between 50 and 70 years. Signals consisted of osteoporotic fracture of the thoracic spine seen on lateral radiographs of the lung. Oral signs were the number of teeth present, cortical thickness, alveolar bone resorption, and

morphological classification of the cortex in the panoramic radiograph. The number of teeth (N) was significantly correlated with the probability of fracture in the thoracic spine and was used to derive equation and the probability for the presence of fractures of the thoracic spine: probability value = 1 / (1 + z), where Z age = 18.68 to 0.29 - 0.27 N. The probability value greater than 0.5 suggests the possibility of fractures in the thoracic vertebrae. It can be concluded that this equation combined with the findings in the panoramic radiograph could serve as a simple and useful tool for the dentist to evaluate the possibility of latent osteoporosis.

Panoramic radiographs are routinely used in most radiographic indications for the various types of dental patients. Such use as the primary complementary diagnostic exam is endorsed by Nº. 453 Law of the Health Ministry - ANVISA – Brazil, a recommendation supported by the principle of radioprotection known as ALARA (As Low As Reasonably Achievable), i.e. we should always use the least amount of radiation possible, for better diagnostic information and for the well-being of the patient (SVS-MS, 1998).

In 1991 an index of bone mass radiomorphometric cortical (BENSON et al, 1991) the panoramic mandibular index (PMI). The MPI was obtained as the result of the ratio between the thickness of the mandibular inferior cortex (ECM) and the distance between the bottom of the mental foramen and the lower limit of the mandibular inferior cortex (DMC). Being that the higher the value of IPM, the lower jaw bone resorption. The differences in the index in a population of 353 adults, evenly divided by gender, age (30 to 90), and racial groups (blacks, Hispanics and whites) were evaluated with respect to the side, race, gender, and age, and combinations of these variables. Blacks were found in average IPM higher than in Hispanics or whites, who were demographically similar. Age-related changes comparing younger and older groups within each sex and racial group showed a significant decrease in average IPM with increasing age in black and Hispanic women. The average PMI in whites increases with advancing age.

The precision of the panoramic mandibular index in detecting patients with osteopenia and osteoporosis was studied and the authors concluded that the action taken in panoramic radiographs (IPM) of the patients studied was able to identify low bone mass and is able to differentiate patients with osteopenia and osteoporosis. Thus, IPM can be used by dentists to make an early approach that osteoporosis is a systemic condition that affects almost half the female population and brings many risks and damage their health (KNEZOVIC-ZLATARIC & CELEBIC, 2005).

Other authors (NAKAMOTO et al, 2003) assessed whether untrained dental practice would be able to determine whether panoramic radiographs women have low bone mineral density (OD). The researchers studied the concordance of the observer and the diagnostic efficiency in detecting low DOM in women. This was done when the appearance (normal or eroded) of the mandibular inferior cortex on dental panoramic radiographs of 100 postmenopausal women who had carried out assessments DOM lumbar spine and femoral neck. The intra-and inter-observer was assessed with kappa statistics. The diagnostic efficiency (sensitivity, specificity, and predictive values) was analyzed by comparing the two groups classified by the mandibular inferior cortex (women with normal cortex and women with eroded mandibular inferior cortex) with those classified by DOM (DOM women with normal and women with osteopenia or osteoporosis). The average sensitivity and specificity were 77% and 40%, respectively, when the DOM of the lumbar spine was used as the default, and 75% and 39%, respectively, when the DOM of the femoral neck

comprised the standard. Nineteen of the 21 untrained general dental practice showed a moderate to almost perfect intra-observer agreement. We conclude that dental panoramic radiographs could be used in clinical dental practice to identify postmenopausal women who have low DOM undetected.

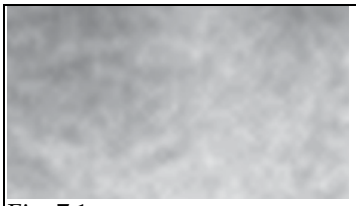
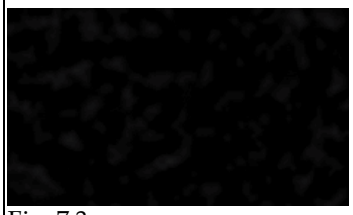
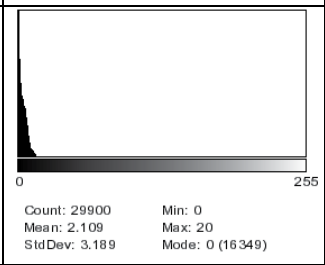
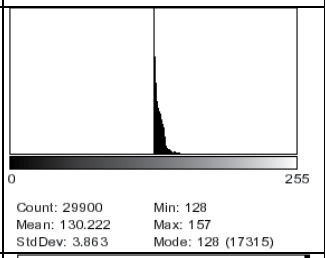
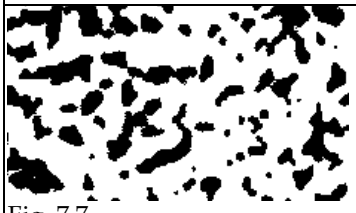
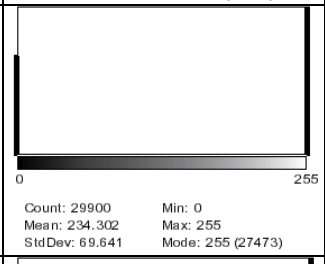
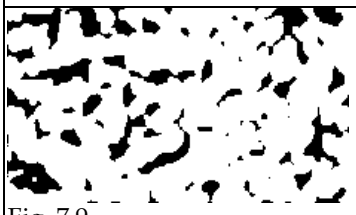
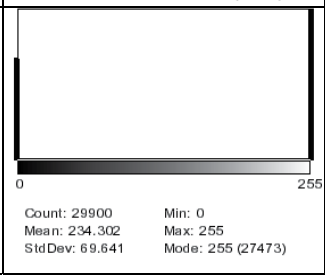
The frequency of osteoporosis was evaluated according to bone sites using a cross-sectional clinical study. The authors evaluated 610 densitometric examinations in relation to frequency of osteoporosis / osteopenia and agreement of the diagnosis according to the bone site. Despite the high correlation of BMD between the different bone sites, the frequency of osteoporosis varied with the site assessed. This study demonstrated that there is discordance in the BMD results according to the study area, affecting the occurrence of osteoporosis. Clinical trial for fracture risk assessment, the use of two different bone sites is the most appropriate procedure. For routine clinical dental surgeon, which includes the panoramic radiography in the care protocol and the jaws could be used for that purpose, and to request carpal radiography, which can add information of bone quality, especially in view of the proportions of trabecular and cortical bone of the phalanges of the hand and also in the cortical distal radio (ZANETTE et al, 2003).

In 2005, Klein conducted a study conducted with the objective to modify the skeletonization algorithm to quantify and create other radiographic images in panoramic radiographs. According to the study of observations and the evaluations that were made, it can be concluded that: 1) the part of the experiment related to the use of radiographic images by means of skeletonization on panoramic radiographs was effective because it increased the radius of the visual perception of the architecture in the trabecular bone and observed the trabeculae, the marrow spaces, such as micro-damage, or micro fractures, 2) despite the agreement between the examiners who have not reached recorded levels above 80%, a high significance in the overall proportion of black points and end points with the odds of a diagnosis concerning the existence or not of bone fragility. 3) results confirmed that the greater bone fragility actually revealed to be a loss of lamellae of trabecular bone architecture and its fairly large marrow spaces.

When studying osteoporosis, there is consensus that inexpensive methods of screening for osteoporosis are needed. The results of this study (WHITE et al, 2005) suggest that dentists have sufficient information to routinely identify people with low BMD using the images of panoramic radiography in dental practice. Radiographs with low doses of radiation, comparable to 4 bitewing radiographs, and the patients identified as having risk of osteoporosis should be referred to a primary health care for further evaluation.

The literature on oral radiographic signs of osteoporosis was revised in 2002, including alveolar bone resorption, and decreased inferior cortical mandibular (ICM). The authors concluded that the panoramic radiograph is an important tool that displays enough information to diagnose osteoporosis (WATANABE et al, 2002). Also in 2002, HORNER et al made a study evaluating the relative utility of clinical indices and radiographic diagnosis in patients with low skeletal bone mass between 135 on healthy pre-menopausal women, aged 45-55 years who sought dental treatment. The DOM was measured in the spine and hip using DXA and classified according to the WHO criteria for Caucasian women. In each patient the (ICM) was measured on panoramic radiographs. The body mass index (BMI) is a simple calculation of the estimated risk of osteoporosis (SCORE). All indexes, (ICM), BMI and SCORE showed significant correlation with skeletal bone density. Thus, the authors concluded that the thinning of the ICM <3mm in peri-menopausal healthy women is associated with low skeletal bone mass.

Three indicators of bone quality on panoramic radiographs were studied to determine the correlation with low DOM using DXA in a Brazilian population (Watanabe, 2003). Examination of the trabecular bone and ICM in the panoramic radiograph showed early signs of osteoporosis. There was significant correlation of these factors with the parameters measured as the percentage of trabeculae, fractal dimension and trabecular connectivity (Figures. 13 and 14).

 Fig. 7.1	Image interest area (original), 230 X 130 *pixels*	 Fig. 7.2	Image copy of the Figure 7.1, with "Gaussian blurr" of 33 radius (pixels)
 Fig. 7.3	Result of subtraction Figures 7.1 and 7.2 images process		Fig. 7.4 Histogram 7.3 image
 Fig.7.5	Result of addition of the constant, 128 to 7.3 image		Fig. 7.6 Histogram 7.5 image
 Fig. 7.7	Result of binary transformation (threshold) of the 7.5 image, with 128 brightness value		Fig. 7.8 Histogram 7.7 image
 Fig. 7.9	Result of the erode process of the image Figure 7.7		Fig. 7.10 Histogram of the image Figure 7.9

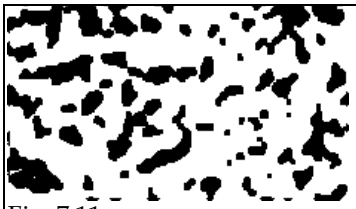


Fig. 7.11

Result of the dilate process of the image Figure 7.9

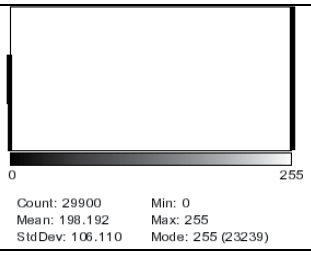


Fig. 7.12 Histogram of the image Figure 7.11

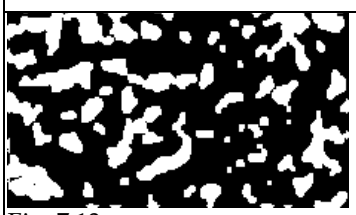


Fig. 7.13

Result of the 7.11 image inversion

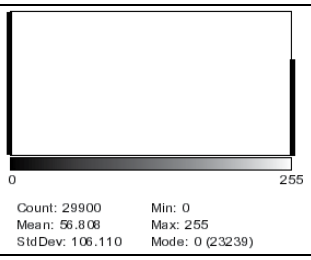


Fig. 7.14 Histogram of the image Figure 7.13

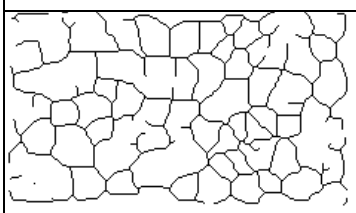


Fig. 7.15

Result of the image esqueletonizing action Figure 7.13

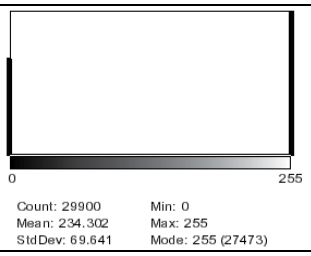


Fig. 7.16 Histogram of the image Figure 7.15

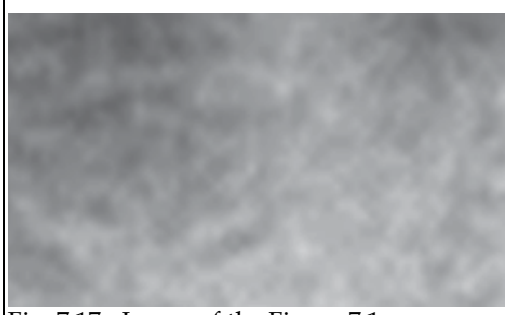


Fig. 7.17 - Image of the Figure 7.1

conclusão

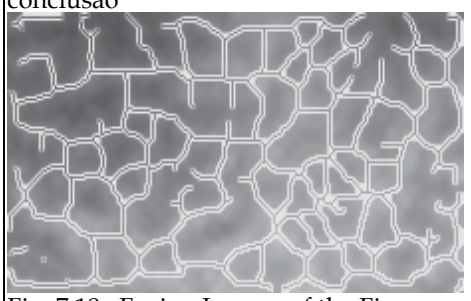


Fig. 7.18 –Fusion Images of the Figures 7.1 e 7.15

Fig. 13. Representation of the process of esqueletonized of region of mandibular interest, panoramic x-ray, for study of the percentage of trabeculae, fractal dimension and bony connectivity.

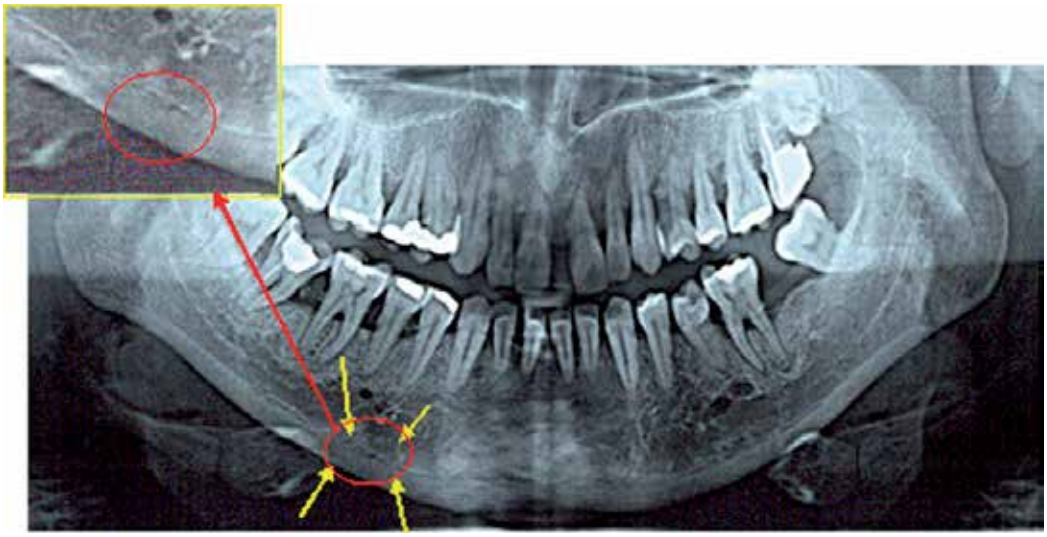


Fig. 14. Detail of the erosion in the mandibular inferior cortical

The diagnostic performance of measurements on panoramic radiographs (PR) and a self-assessment tool for osteoporosis (OST) that identifies women with spinal osteoporosis (WHO) on 159 pre-menopausal (PrM) and 157 post-menopausal (PsM) women with a history of hysterectomy, ovariectomy, or use estrogen were compared. The morphology of the ICM and its thickness were evaluated in the panoramic radiographs. The authors concluded that clinicians can refer women with suspected osteoporosis (PsM) in the column to undergo DXA based on tests conducted on panoramic radiographs with similar performance in the OST (TAGUCHI et al, 2004). The correlation of Klemetti rating for the PR using digital panoramic radiographs of Brazilian women was also studied in 2004 by TAGUCHI et al. Significant correlation was found with DXA of the forearm DOM, indicating that the panoramic radiograph is valuable in the identification of patients at risk for osteoporosis.

DOM and radio-morphometric linear parameters in elderly patients with different types of dentures were studied (DUTRA et al, 2005). Three parameters were measured: Mandibular inferior córtex thickness (CIMT), the Antegoniac Index (IA) and Goniac Index (GA). The DOM was measured in the jaw with the use of a copper penetrometer. The results showed that there was a statistically significant difference between patients with all teeth and those with denture for all the measured radio-morphometric indices ($p < 0.001$). Also in 2005, LEE et al suggested that dentists had sufficient radiographic and clinical information to identify patients with osteoporosis. The author concluded that the changes found in the trabecular structures on panoramic radiographs supplemented with clinical information is an indicative of risk for hip fracture in elderly women.

It can be concluded that the dentist may suspect systemic risk of osteoporosis when the patient presents the following radiographic signs found in panoramic and periapical radiographs (WATANABE et al, 2004):

- Klemetti Class II or III, radiolucent spaces in the mandibular inferior cortical (Fig. 10);
- CIM thickness less than 3.0 mm;
- Disorganization of the basal mandibular trabecular bone with low numbers and low connectivity;

- sharp contrast between the branch / mandibular body and strengthening of structures, such as the oblique line (Figure 15).

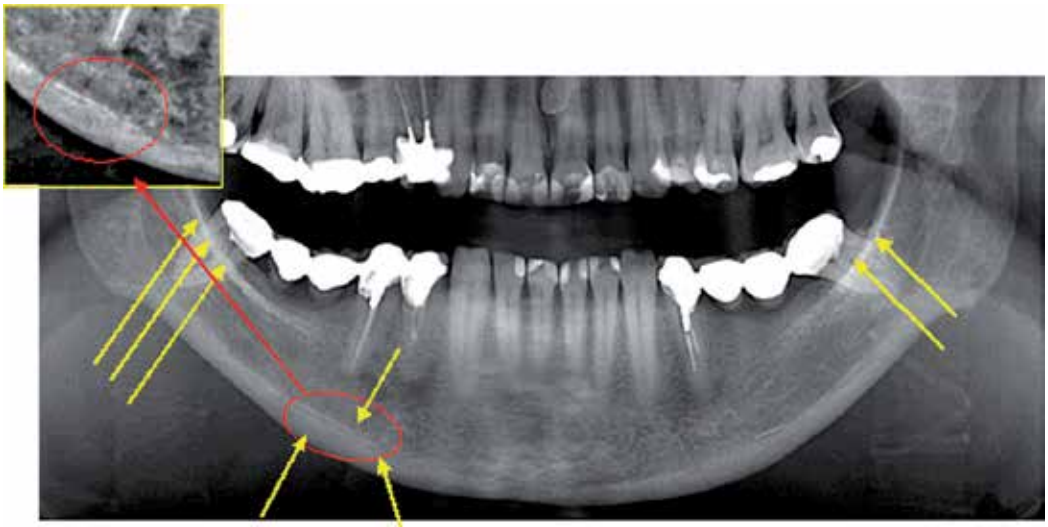


Fig. 15. Detail of the pseudoperoartrite in the mandibular inferior cortical and accented oblique line in the mandible.

It is possible to correlate Bone Mineral Index (BMI)with mandibular bone quality (MBQ). The authors studied the correlation between body mass index and mandibular bone quality in Brazilians of both sexes. According to the methodology employed in this study, not all patients with poor MBQ had low BMI, but the majority who had low BMI, had bad MBQ (DUTRA et al, 2005).

$$IMC = \frac{Weigth(kg)}{Heigth^2(m^2)}$$

Underweight	Normal	Overweight	Obese
<22	22,0 - 24,9	25,0 – 29,9	≥ 30,0

Lee et al (2005) conducted a study in the visual cortical lower mandible on panoramic radiographs to identify postmenopausal women with low BMD. The authors concluded that the visual analysis of the mandibular inferior cortex on panoramic radiographs may be useful in identifying women with low DOM PM (LEE et al, 2005).
Dutra et al studied the radiomorphometric indices and their relationships with gender, age and dental status, using the antigoniac index (AI) and chin index (MI) in patients with and without teeth. It was concluded that there is a renovation in the mandibular inferior cortex (MIC) with age and that would be influenced by gender and dental status. The difficulty in measuring the AI in a reproducible way, and their interaction with dental status and low correlation with MI in younger patients would discourage its use for the purpose of identifying patients at risk of osteoporosis ((DUTRA et al, 2005).
An image analysis software that can accurately measure the thickness of the mandibular inferior cortex (MIC) in PR has been developed as an indicator of low BMD. The authors found that the action taken by the software had significant correlation with the BMD and could contribute to the identification of osteopenia. The study was supported by the European Commission FP5 "Quality of Life and Management of Living Resources"(Report

of the 10th European Congress of Dentomaxillofacial Radiology, 2006). Another study investigated the OSTEODENT trabecular pattern in intraoral radiographs, and concluded that this factor would serve for the diagnosis of osteoporosis. Other authors stated that ICM is effective in the diagnosis of osteoporosis because it had high specificity and thus could be used in primary health care. Analyzing the densitometric measurements in intraoral radiographs to detect osteoporosis was the proposal of some authors who concluded that bone density in the region of premolars, expressed in millimeters of aluminum would be favorable for showing the presence of systemic osteoporosis.

In 2007, Ishii et al evaluated the diagnostic efficiency in identifying postmenopausal women with osteoporosis by analyzing femoral bone loss in the jaw. It is known that cortical thickness measurements in lower jaw is useful to that purpose. The results suggest that the assessment of alveolar bone resorption was not as effective in detecting postmenopausal women with osteoporosis compared to femoral cortical thickness measures lower jaw.

The detection of cortical erosions in lower mandible on panoramic radiographs and tools based on questionnaires were studied in 2008, and it was found similar diagnostic efficacy in identifying postmenopausal osteoporotic women. Furthermore the authors evaluated the diagnostic performance to identify osteoporosis and biochemical markers of bone turnover for high risk of fracture. The analysis of urine and blood plasma were measured for bone mineral density (OD) of the spine and hip by DXA. The results suggest that panoramic radiography was superior to questionnaire-based tool to identify women with high risk of fractures (TAGUCHI et al, 2008).

In 2009, Elsubeihi & Heersche studied the effects of ovariectomy on the toothed jaws and mandible of rats were investigated and compared to changes in relation to the tibia and femur using DXA scans (dual X-ray Absorptiometry) and histomorphometric measurements. The results showed that the loss of bone in the jaws without teeth in ovaryectomized animals was similar to what occurred in the tibia and femur, while the lack of significant effects of ovariectomy on bone mass in toothed mandibles suggests that the functional load on the force bite prevents bone loss in the jaws with teeth. Evaluating the quality of mandibular bone in edentulous persons, persons with less than 21 teeth and those over 21 teeth, it was found significant differences in the bone quality, measured by the mandibular inferior cortical thickness, indicating that the person with more than 21 teeth in the oral cavity has a better quality of mandibular bone.

It was also studied in 2010, by Watanabe et al, the correlation of the elongated styloid process with low BMD diagnosed by DXA. The authors could verify the existence of a strong correlation between women with osteopenia and osteoporosis, and women with fracture risk presented calcification of the stylohyoid ligament (Figure 16). In another article of the same year, Watanabe et al, 2009 studied the morphological pattern trabecular digitally comparing the same regions of interest in different radiographs, periapical and panoramic (Figure 17). The authors could verify that when the analysis of skeletonized images of certain regions was performed, significant differences between the measurements were found and such comparison should therefore be carefull. Khojastehpour et al. analyzed the usefulness of the Panoramic Mandibular Index (PMI) on panoramic radiographs for the diagnosis of osteoporosis in women and concluded that dental panoramic radiographs could be used in the clinical practice to assist identifying individuals with low bone mass through PMI (KHOJASTEHPOUR et al, 2009).

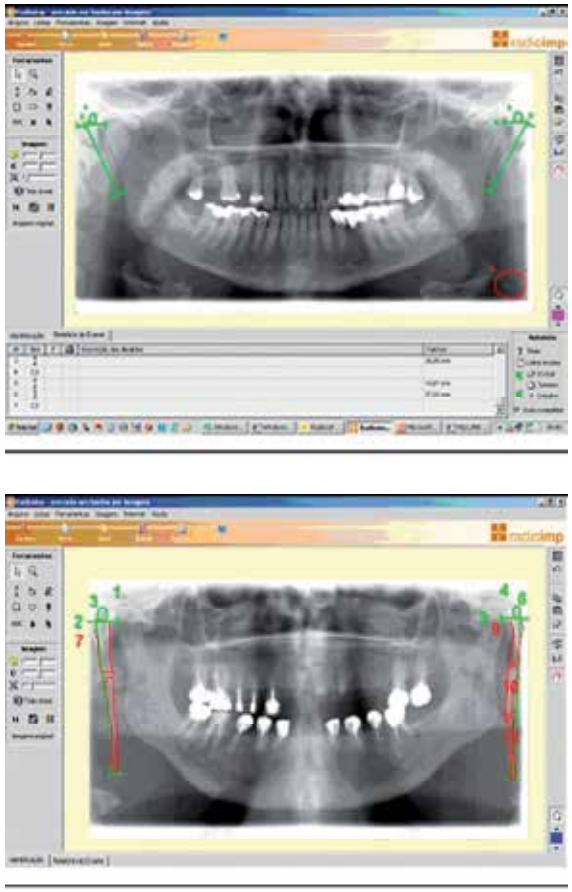


Fig. 16. Measure of the Elongated styloid process in the panoramic radiographic

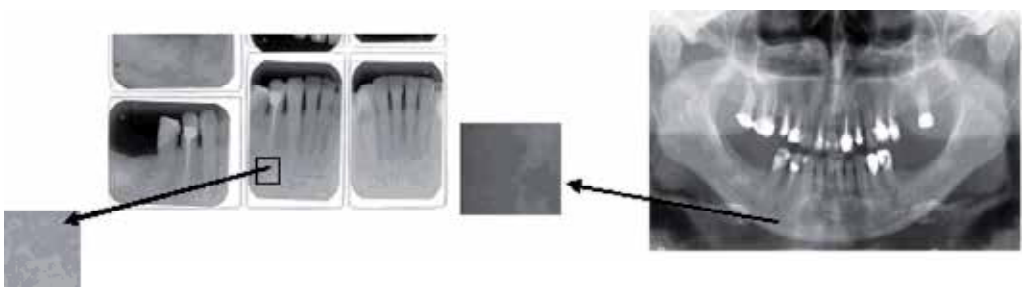


Fig. 17. The same interest region to prepare the image to skeletonized

8. Periodontal disease and osteoporosis

Some studies have suggested that osteoporosis and periodontitis are associated (PERSSON et al, 2002): (1) the prevalence of self-reported history of osteoporosis in an older population, ethnically diverse, (2) the concordance between panoramic mandibular index (PMI) and self-reported osteoporosis, and (3) the probability of having a self-

reported history of osteoporosis and a diagnosis of periodontitis. Panoramic radiographs and medical histories were obtained from 1084 Chinese women aged 60-75 years (mean ± 68.5 years). Patients were classified as having or not periodontitis or within three grades of severity. The PMI was found positive in 39% of patients, in contrast to self-reported osteoporosis (8%). The intra-class correlation between the PMI and self-reported osteoporosis was 0.20 ($p < 0.01$). The probability of an association between osteoporosis and IPM was of 3%. Patients with osteoporosis and self-reported a positive PMI had worse periodontal conditions ($p < 0.01$).

The prevalence dominance PMI positive was high and consistent with the epidemiological studies however, only partly consistent with a self-reported history of osteoporosis, with a higher prevalence of positive PMI. The loss of horizontal alveolar bone was associated with osteoporosis and self-reported positive results of PMI. Contradictory findings were found by authors (LUNDSTROM et al, 2001) who examined periodontal conditions in a cohort of women aged 70 years compared with an osteoporotic flu control with a normal BMD (210 women, 70 years). Hip radiographs were measured with DXA. The examination included a PR and intraoral radiographs. In conclusion, the study found no statistical significance in the periodontal conditions or marginal bone level between the two groups, although the results should be interpreted with extreme caution as the study sample was small.

We studied the correlation between periodontal disease and osteoporosis, comparing age, parameters of the panoramic radiographic and clinical periodontal disease. The panoramic radiographic parameters evaluated were: mandibular cortical thickness (MCT), patients were not treated, adults who had no other systemic disease and should have more than 20 teeth. They were evaluated by panoramic radiography with respect to alveolar bone loss (ABL). The mandibular bone mass was assessed by measuring the mandibular inferior cortical thickness (MICT). The POA was significantly higher CIMT and significantly lower for patients PMs (> 6 years after menopause). The number of teeth was significantly lower in the group PM (> 11 years after menopause). The age and ABL had positive correlation in men and women. Women in which MICT was lower than the average (- 2 SD) should be diagnosed as osteoporosis. The results showed that periodontal disease has correlation with osteoporosis, and thus the MICT could be useful in detecting signs of osteoporosis in women with periodontal disease (OTOGOTO & OTA, 2003).

Some authors (JAGELAVICIENE & KUBILIUS, 2006) evaluated the relationship between systemic osteoporosis and periodontal disease. Radiology provides information in determining the type and degree of alveolar resorption, periodontal condition, and the number of teeth. These parameters provide valuable information when the corresponding data correlation study was searched.

8.1 Osteonecrosis of the jaw after oral bisphosphonate for osteoporosis

Although all the benefits of the therapy with bisphosphonate, mainly for the treatment of osteoporosis, this drug is commonly associated with osteonecrosis of the jaw (ONJ). The use of bisphosphonate was first reported in 2003 (MARX, 2003), and other case series reported similar findings, usually in patients undergoing parenteral treatment for malignancies. Osteonecrosis of the jaw associated with the use of oral bisphosphonate for the treatment of osteoporosis is much less frequently reported (PAZIANAS et al, 2007; YARON et al, 2007). The American Society for Bone and Mineral Research defines bisphosphonate-associated ONJ or BRONJ as “an area of exposed bone in the maxillofacial region that has not healed

within 8 weeks after the identification by a healthcare provider in a patient who is receiving or has been exposed to a bisphosphonate and has not had radiation therapy in the craniofacial region" (KHOSLA et al, 2007) . The American Association of Oral and Maxillofacial Surgeons (AAOMS) (WOO et al) has revised its 2006 landmark position paper on Bisphosphonate-Related Osteonecrosis of the Jaw to reflect the most current research on this condition. BRONJ appears as a non-healing exposed bone in the maxillofacial region and may affect patients undergoing intravenous cancer-related bisphosphonate therapy or more rarely, patients treated with oral or IV bisphosphonates for osteoporosis. Despite its low prevalence, the potential risk of BRONJ occurring after the use of oral bisphosphonate for osteoporosis should never be neglected.

Also, the European Society on Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) has developed some standards for the prevention, recognition and management of BRONJ (PHAL et al, 2007). In osteoporosis patients, no specific interventions prior to starting bisphosphonate therapy are required except to encourage regular dental care.

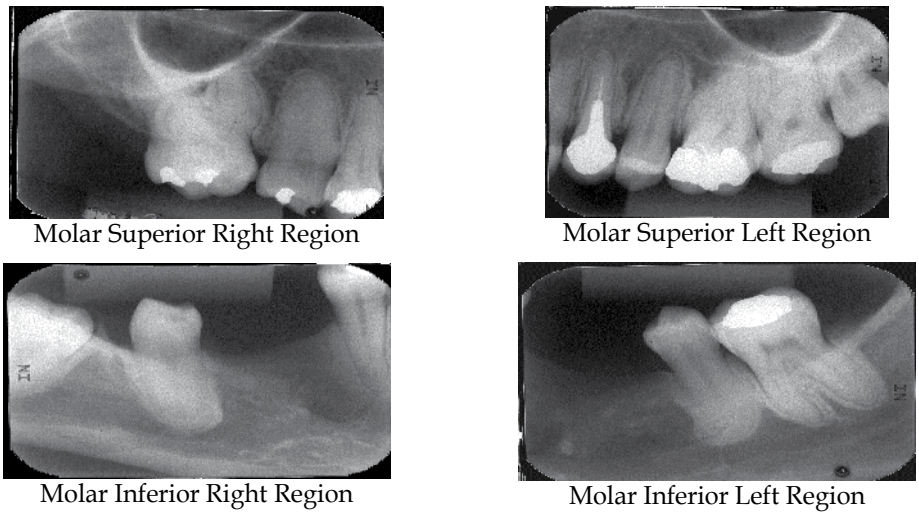
The radiographic aspects associated with osteochemonecrosis of the jaws related with the oral use of bisphosphonate (BP) should be identified. The most important radiographic features are: Osteosclerosis limited to the alveolar process, widening of the lamina dura, expansion of the periodontal ligament space, bony sequestra, jaw expansion, radiolucency, and periosteal new bone formation. Osteosclerosis is frequent. It is usually found in the presence of periodontal disease, probably because it is attributed to the fact that BP accumulates preferentially in sites of high bone turnover or remodeling (KHAN et al, 2008).

Radiographic changes are not evident until there is extensive bone involvement. Therefore, panoramic radiographs may not reveal significant changes in the early stages of osteonecrosis as numerous different patologies. When there is extensive bone involvement, regions of mottled bone similar to diffuse osteomyelitis or postirradiation osteoradionecrosis are noted (KUNCHUR & GOSS, 2008). After prolonged exposure to intravenous bisphosphonates, osteosclerosis of the bone, especially osteosclerotic lamina, may be noted radiographically Edwards et al, 2008 (Figures 18-19). The patient in these images received the diagnosis from breast cancer IIIa - AP, and began chemotherapy in July 2003, and gone through bilateral mastectomy. In 2007 it had been diagnosised metastasis in the liver and in the column when the x-ray of the column was made and the monthly use of zometa. The first requested x-ray to the Radiologic Service was in 2008 for the Dental Service of the hospital for diagnostic purpose. It was observed the absence of 7 teeth and horizontal resorption of the alveolar Crests and sclerosis of the horny lamina of teeth 16; 15; 14; 13; 25; 27; 36; 35; 45 and 48. These alterations are indicative of possible bone exposition, suggesting the presence of a sub-clinic degree of osteonecrosis, "the zero" in accordance with AAOMS. In April of 2009, new panoramic x-ray was made and served as base for dentists to consider the interruption of the administration of zometa. After the evaluation of the patient, physicians had substituted zometa by 70mg of alendronate sodium, in an attempt to prevent the occurrence of osteonecrosis in the maxillaries. The jaws are mainly affected because of the teeth, that are embedded in bone. This active bone has fast turnover, principally the horny lamina (alveolar bone also). Most of the times, dentists need to proceed with invasive dental procedures that injures the bone. Hence, the osteonecrosis is viable.

8.2 Incidence

- 0.8% -1.6% (industry-sponsored)
- 8% -12% (independent)
- AAOMS

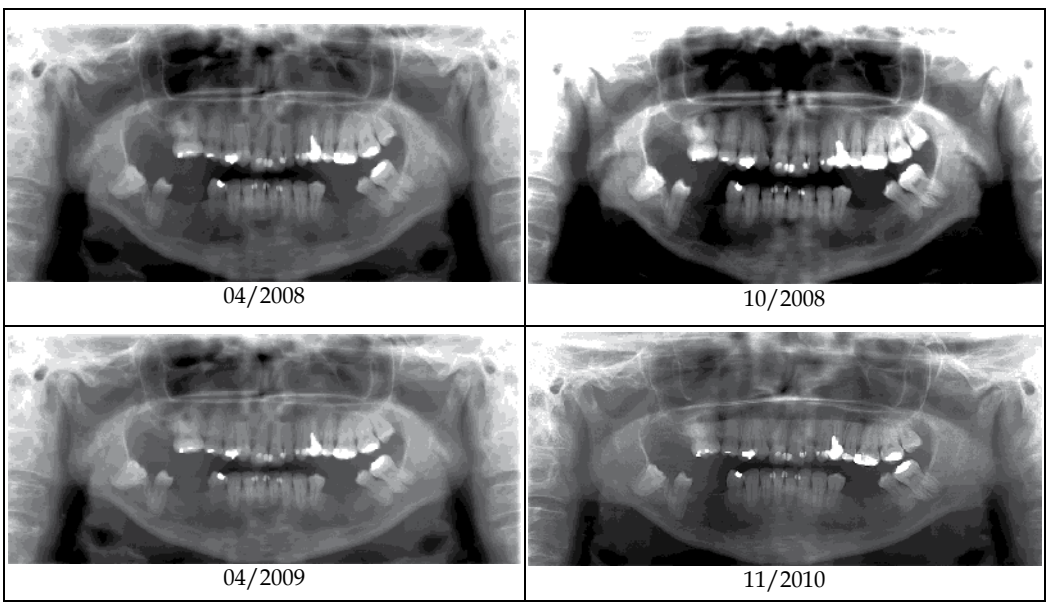
0.01% -0.04% (oral) 0.8% -12% (i.v.)



Molar Superior Right Region Molar Superior Left Region

Molar Inferior Right Region Molar Inferior Left Region

Fig. 18. Periapical radiographs showed widening of the lamina dura in all regions in the mouth



04/2008 10/2008

04/2009 11/2010

Fig. 19. Panoramic radiographs showed widening of the lamina dura in all regions in the mouth in four differents periods.

9. Conclusions

Dentist are healthcare professionals and currently graduate with a different vision for prevention. Modern dentistry has been made responsible for important technical and socio-economic status. New technologies and treatments developed brought great advances in improving the health of the population. Visits to the dentist are much more frequent, and it is routinely visited by patients who have never had cavities. Elucidation of the population with respect to dental care has turned dentists in a professional for oral diagnosis, monitoring, prevention and oral aesthetics, rather than a curative professional.

This expanded the role of dentistry, including the Family Health Program (FHP) in Brazil that is the most significant advancement of the profession, which leads us to think about how these professionals can contribute to the improvement of health as a whole, enabling the dentist to act more widely.

The dentist is who examines the mouth of the population. The teeth are only a portion of the mouth. There are a huge range of other elements that require constant care and observation. Thus, the saying "dentistry beyond the teeth" reinforces, and aims to modernize dentistry career on several fronts, giving a broader professional training and creating conditions so that they can increasingly contribute in improving the health of the population. This modernization involves different aspects ranging from a reformulation, modification of certain areas of research within the faculties, until a fight over a new aspect of insertion of the dental professional in the job market.

We feel that there is sufficient evidence that the radiographic images that the dentist routinely uses, particularly the panoramic radiograph can provide important signals related to poor bone quality, and thus we suspect that the involvement of other bone sites such as spine, hip and forearm, and sites that increase the risk of osteoporotic fracture. We therefore endorse the patients with poor bone quality diagnosed by the oral physician to search for other skeletal sites for poor bone quality. Early detection can lead to appropriate treatment and relief of adversities. This is an area where the dentist can greatly contribute in reducing the morbidity and even the mortality, thus enhancing its performance as a health professional, understanding the patient as a whole.

As osteoporosis is a global epidemic with enormous social costs, with high morbidity and mortality; the Global Forum 2005 indicated osteoporosis as a neglected disease. These are the diseases that, despite having a high incidence in the developing countries, do not receive investments in Research & Development in proportion to their epidemiological importance. Neglected diseases can be defined as a group of diseases associated with poverty. The precarious living conditions and health inequities are major factors responsible for the incidence of neglected diseases. So it is important that the Dentist be prepared for the possibility of evaluating and interpreting the morfometric indices on panoramic radiographs, which could allow the interaction with other health professionals in assessing and preventing the risk for osteoporosis.

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Effect of Bisphosphonates on Root Growth and on Chlorophyll Formation in *Arabidopsis thaliana* Seedlings

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1. Introduction

Bisphosphonates (BPs) are analogues of pyrophosphate in which the oxygen bridge between the two phosphates is replaced by a methylene group (–CH_2–). Substitution of one or both hydrogen atoms of this group by radicals generates a variety of bisphosphonates; usually they are grouped into two types, namely non-N-BP and N-BP, depending on the absence or presence of a nitrogen atom in its molecule, respectively (Russell, 2011). Examples of BPs used in this report are: etidronate and clodronate (non-N-BPs), and pamidronate and alendronate (N-BPs).

Bisphosphonates are the leading drug class for the treatment of osteoporosis; as an indication of their usefulness and the spread out of the disease the combined sales of drugs to treat osteoporosis reached $ 6.2 billions in 2004. Besides their application in humans, bisphosphonates are also used for other purposes, mainly as herbicides in plants (Cromartie et al., 1999; Oberhauser et al., 1998), as chemotherapeutic agents (Artz et al., 2008; Docampo & Moreno, 2001; Leon et al., 2006; Moreno & Li, 2008) and in basic research (Rogers et al., 2010; Russell, 2011).

The usefulness of bisphosphonates is due to their mechanism of action when they are supplied to the living organism. In fact, they have the ability of interfering with metabolic pathways located at the crossroads of essential processes for life. Some important examples of this crucial role are as follows:

a) Bisphosphonates may act as analogs of PP in many of the reactions catalyzed by ligases and some transferases (reaction 1) in which derivatives of the type NRpp-CH_2-p are synthesized in the reverse reaction (reaction 2) (Günther Sillero et al., 2008; Günther Sillero et al., 2006; Rogers et al., 1996; Russell, 2011).

$$X + NRPPP \text{ ----> } X\text{-}NRP + PP \quad (1)$$

$$X\text{-}NRP + p\text{-}CH_2\text{-}p \text{ ---> } NRpp\text{-}CH_2\text{-}p \quad (2)$$

$$X\text{-}NRP + mev\text{-}pp \text{ --->} mevpppN \quad (3)$$

Where X is a potential substrate of the reaction; NRP and NRPPP are nucleosides mono- and triphosphates, respectively; NRpp-CH_2-p is a nucleoside 5′(β,γ methylene triphosphate) and mev-pp a compound of the mevalonate pathway with a terminal pyrophosphate (see below). As the more abundant nucleoside triphosphate is ATP, the more common derivative from bisphosphonate, would be ARpp-CH_2-p; given that ATP is a co-substrate of at least 150 enzymes with transferase activity and more than 90 with ligase activity (see Enzyme Nomenclature), bisphosphonates may indirectly interfere with multiple cellular processes through the formation of bisphosphonate derivatives of ATP or of any nucleoside triphosphate. As in these reactions BPs act as analogs of PP it would be expected that the non-N-BP (the smallest type of BP) were the preferred substrates for these reactions.

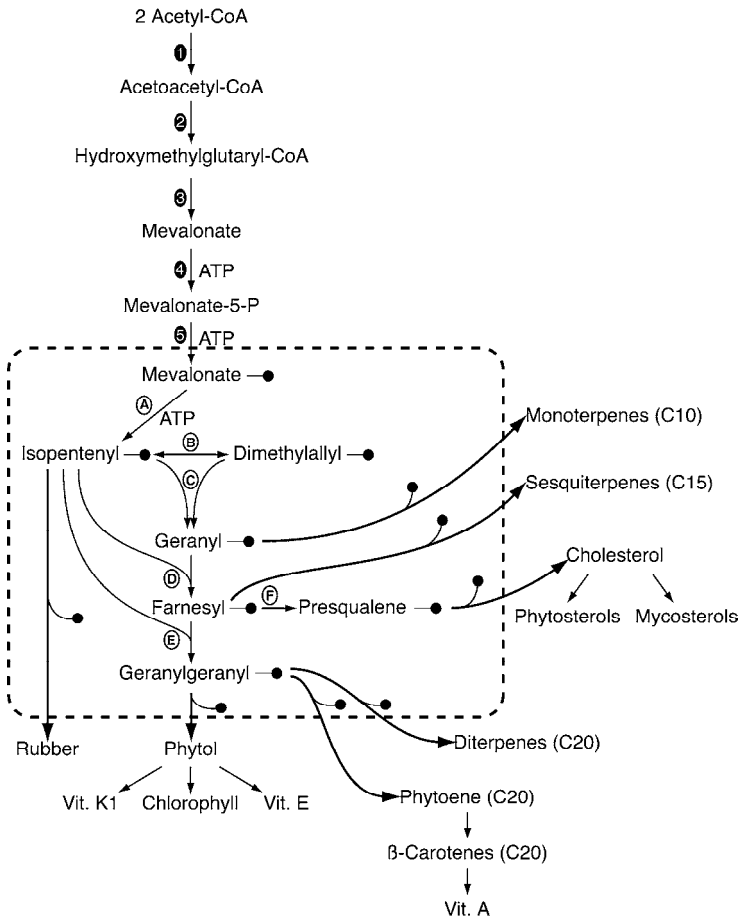
b) BPs may be inhibitors of enzymes having substrates with a terminal PP (R-PP) (see below and Günther Sillero et al., 2009). Although the inhibition of these enzymes could take place at any step of the pathway, specific inhibitions have been reported on isolated enzymes from bacteria, yeast or plants: pyrophosphatase (Baykov et al., 1993; Cromartie et al., 1999; Drozdowicz et al., 2003; Gordon-Weeks et al., 1999; Kim et al.,1994; Kuo et al., 2005; Rodrígues et al., 2000; Szabo & Oldfield, 2001; Zhen et al., 1994); geranyl diphospho synthetase (Burke et al., 2004; Oberhauser et al., 1998); isopentenyl pyrophosphate synthase (Cromartie et al., 1999) and P5C reductase (Forlani et al., 2008).

c) The synthesis of isopentenyl triphosphoadenosine (iso-pppA) was previously described (Monkkonen et al., 2006). Following this finding, the synthesis of derivatives of a variety of compounds of the mevalonate pathway capped with an adenosine moiety catalyzed by several ligases, was later reported (mev-pppA and mev-ppppA) (Günther Sillero et al., 2009). Increase in the concentration of metabolites upstream the inhibited step could stimulate synthesis of the corresponding mevalonate derivative (reaction 3) (Günther Sillero et al., 2009; Rogers et al., 2010).

Related to the use of bisphosphonates in humans for the treatment of osteoporosis, Paget's disease and bone tumour metastasis it can be stressed that upon their oral or intravenous administration BP are partially eliminated by kidney and partially fixed in bones, with very little amount in the systemic circulation (Cremers et al., 2005). Being otherwise bisphosphonates could have general toxic effects. After their capture by osteoclasts from the bone they exert noxious effects on these cells by some of the above-mentioned mechanisms. The basic approach, necessary to explore the effect of BPs, is not sufficient to envisage their clinical effects. These studies, although mandatory, are cumbersome, costly and requiring a cohort of patients as each patient has distinct characteristics and each bisphosphonate may present special pharmacokinetic properties (Cremers et al., 2005).

Based on the above we thought of interest to approach the effect of bisphosphonates in plants: plants are a whole living entity, of cheaper handling, where BPs present a quite different pharmacokinetics; they are easily assimilated by the roots, are not fixed in a structure similar to bones and probably circulate through the vegetal tissues more easily than in animal tissues. As an initial approach to the problem, we have used germinating seeds and early seedlings of the model plant species *Arabidopsis thaliana* to investigate the alteration of a few phenotypic characters, considered of high relevance for key functions of the plant, namely the seed germination rate, the early development of primary and secondary roots (essential for establishing the plant developmental pattern) and the presence and content of chlorophyll in the first leaves. This later character, used as an indicator of the ability of plants for performing the fundamental process of photosynthesis, also indicates the alteration of the mevalonate pathway, since chlorophyll is a product of

this biosynthetic pathway. We have focused our attention on this pathway as a good target to examine the effect of BP given the two metabolic peculiarities present only in the mevalonate cycle: occurrence of both three consecutive enzymes requiring ATP as a co-substrate and the major pool of compounds containing a terminal pyrophosphate in any known metabolic pathway (Günther Sillero et al., 2009) (Fig. 1).



The metabolic pathway from acetyl-CoA to mevalonate-5-phospahte, and the implicated enzymes (1-5) are indicated in the figure. The enzymes are: 1, acetyl-CoA acetyltransferase (EC 2.3.1.9); 2, hydroxymethylglutaryl-CoA synthase (EC 4.1.3.5); 3, 3-hydromethylglutaryl-CoA reductase, (EC 1.1.1.34); 4, mevalonate kinase (EC 2.7.1.36); 5, phosphomevalonate kinase (EC 2.7.4.2). Note that the three consecutive steps catalyzed by enzymes (4), (5) and (A), consume three moles of ATP. The inset in the figure comprises the compounds of the mevalonate pathway and the enzymes (A-F) involved: A, diphosphomevalonate decarboxylase, (EC 4.1.1.33); B, isopentenyl-diphosphate D-isomerase, (EC 5.3.3.2); C, dimethylallyl*trans*transferase, (EC 2.5.1.1); D, geranyl*trans*transferase (farnesyl-diphosphate synthase), (EC 2.5.1.10); E, farnesyl*trans*transferase, (EC 2.5.1.29); F, farnesyl-diphosphate farnesyltransferase, (EC 2.5.1.21). To emphasize the occurrence of pyrophosphate in all the compounds of the cycle, this moiety has been marked out (•). Some of the great variety of compounds synthesized from those metabolites, and essential for plant functions, is annotated. The mevalonate independent pathway is not represented in the Figure.

Fig. 1. Components of the mevalonate pathway, its precursors and some metabolic connections in plants.

The cytosol-located mevalonate pathway (starting with 2 moles of acetyl-CoA and ending in geranygeranyl pyrophosphate (Fig. 1)) is the starting point for the synthesis of a great variety of compounds in bacteria, plants and animals, embracing more than 23,000 currently identified compounds (Wendt & Schulz, 1998). A different additional route for the synthesis of isopentenyl pyrophosphate is localized in plant plastids. This mevalonate-independent pathway starts with the condensation of pyruvate and D-glyceraldehyde 3-phosphate and, through the action of seven additional enzyme activities, the synthesis of isopentenyl pyrophosphate is obtained (see (Hunter, 2007) for a review).
This short work is an initial report of this study, showing that the plant growth and development, as well as the photosynthetic efficiency, are indeed affected by the presence of BPs in the culture medium, in some cases in such an extent that the actual viability of the plant is compromised. Apart from its basic interest, this finding could be of some help to understand the role of bisphosphonates in animal tissues.

2. Materials and methods

The following bisphosphonates were used in this study: alendronate, pC(OH)($(CH_2)_3$-NH_2)p (Ref. A-4978) and etidronate, pC(OH)(CH_3)p (Ref. P-5248) were from Sigma; clodronate, pCCl_2p (Ref. 233183), and pamidronate, pC(OH)($(CH_2)_2$-NH_2)p, (Ref. 506600) were from Calbiochem. In addition, sodium pyrophosphate and tripolyphosphate (P_3), used in controls, were from Sigma (Refs P9146 and T-5633, respectively).
Seeds of *Arabidopsis thaliana* ecotype Columbia were placed in Petri dishes containing Murashige and Skoog's (MS) medium (Duchefa) in agar supplemented with one of the four bisphosphonates (BPs) indicated, at a concentration of 0.05 mM, 0.1 mM or 0.5 mM. Controls consisted of culture medium alone, or supplemented with pyrophosphate (0.1 mM or 0.5 mM), or tripolyphosphate (0.1 mM or 0.5 mM). Plates were placed vertically and incubated at 22ºC under illumination with a flux of photo synthetically active photons of 150-µE m^{-2} s^{-1}, with a photoperiod of 16 hours of light and 8 hours of darkness. Samples were photographed at 4, 8 and 11 days of incubation and analyzed for the following parameters: a) rate of germination; b) length of the primary root; c) time of appearance, number and length of secondary roots; and d) colour of leaves.

3. Results and discussion

The plants were analyzed by the rate of seed germination and, in germinated seedlings, by the occurrence and aspect of three visible phenotypic characters: growth (length) of the primary root, development of secondary roots (time of appearance, position, number and length) and colour of the leaves, indicative of the presence of chlorophyll. The full set of results obtained for every evaluated parameter with the four BPs, at the three concentrations and in the three time points of sampling assayed, is shown in Table 1.
In order to facilitate the presentation of the results, the experimental conditions have been numbered from 1-12 (#column at the right in Table 1). Furthermore, representative results corresponding to *A. thaliana* seedlings grown during 4, 8 and 11 days in the presence of 0.1 mM etidronate; 0.05 mM clodronate; 0.5 mM pamidronate and 0.5 mM alendronate are shown in Fig. 2. Controls grown without any addition to the culture medium (a-c), or in the absence of BPs, but in the presence of pyrophosphate (d-f) or tripolyphosphate (g-i), were also included in Fig. 2.

NAME OF BISPHOSPHONATE Parameter evaluated	Treatment									
	0.05 mM			0.1 mM			0.5 mM			
	4 days	8 days	11 days	4 days	8 days	11 days	4 days	8 days	11 days	#
ETIDRONATE (non-N-BP): Primary Root Length (Shortening)	+	++	+++	–	–	–	+	+	++	1
Secondary Roots: Reduction in Number and Length	na	na	+++	na	na	+++	na	na	++	2
Chlorophyll Loss	–	–	–	–	–	–	–	–	–	3
CLODRONATE (non-N-BP): Primary Root Length (Shortening)	++	+++	+++	+	++	+++	+	++	+++	4
Secondary Roots: Reduction in Number and Length	na	na	+++	na	na	+++	na	na	++	5
Chlorophyll Loss	–	–	–	–	–	–	–	+	+	6
PAMIDRONATE (N-BP): Primary Root Length (Shortening)	–	+	++	–	+	++	++	+++	+++	7
Secondary Roots: Reduction in Number and Length	na	na	+	na	na	+	na	na	+++	8
Chlorophyll Loss	–	–	–	–	–	–	+	++	+++	9
ALENDRONATE (N-BP): Primary Root Length (Shortening)	–	+	++	+	++	++	++	+++	+++	10
Secondary Roots: Reduction in Number and Length	na	na	–	na	na	+++	na	na	+++	11
Chlorophyll Loss	–	–	–	–	+	+	–	+++	+++	12

The expressed results are referred to the control experiment performed with standard culture medium, without the addition of any other substance. Number of (+) indicates deviation with respect to the control experiment for the particular parameter evaluated. (–): Similar to the control experiment. na: Not applicable, because secondary roots were not initiated in the corresponding control samples. #: Reference number of the experiment, attributed for the sake of clarity in the description and discussion of results.

Table 1. Summary of results obtained on *Arabidopsis thaliana* seedlings with the four bisphosphonates analyzed, the three parameters evaluated and the nine conditions used, resulting from the combination of three concentrations and three times of incubation for each bisphosphonate.

In this initial approach, the estimation of the effects of BPs on the characters investigated has been performed in a semi-quantitative way, by recording the differences that can be appreciated in these parameters by a simple visual inspection. The purpose of this type of analysis was, firstly, to determine whether or not BPs are potent inhibitors of essential functions of the plant; secondly, to discriminate differential effects between the analyzed BPs, the concentrations used and the times of development, in order to select targets for a more detailed and deep mechanistic analysis that may provide relevant information on the specific biochemical reactions that become affected during the inhibitory process.

From the full set of data presented in Table 1, and the selected images shown in Fig. 2, the most relevant results of our study are as follows:

a) None of the tested BPs, at any of the concentrations assayed, affected the seed germination rate. b) Clodronate, pamidronate and alendronate affected both the growth of the primary root, resulting in its shortening, and the number and length of secondary roots (Table 1 and Fig. 2, m-o; p-r; s-u). c) Two BPs, belonging to the same group of non-N-BP (etidronate and clodronate) showed effects of quite different intensity on root development, and might have a different degree of action (Fig. 2, j-l; m-o) (Table 1, compare 1-3 and 4-6). d) The effect of BPs is time dependent, and the highest effect was obtained after 11 days in culture, irrespective of the concentration used. In few cases we observed that the maximum effect was already reached after 8 days and then it persisted after longer time in culture (Fig. 2, n-o; t-u) (Table 1, line 4, 0.05 mM; line 7, 0.5 mM; line 10, 0.5 mM). e) The effect of BPs regarding the alteration of primary root growth was concentration dependent. This feature was more clearly observed with N-BPs rather than with non-N-BPs. However, in the particular case of etidronate, concentrations of 0.05 mM seemed to be more effective than 0.1 mM (Table 1, line 1), a striking result of difficult interpretation. f) There are visible differences on the influence of BPs on chlorophyll loss: non-N-BPs either did not affect visibly that parameter, even at 0.5 mM and during 11 days in culture (etidronate; Table 1, line 3), or affected only slightly at the highest concentration (clodronate; Table 1, line 6); on the contrary, N-BPs, such as alendronate (Table 1, line 12) and to a lesser extent pamidronate, greatly affected the colour of the leaves (Table 1, line 9) (Fig. 2, p-r; s-u). In general, conspicuous effect on chlorophyll loss was only detected at the highest BP concentrations. g) Alendronate was the most effective BP acting on *Arabidopsis thaliana* development, affecting the primary and secondary roots and chlorophyll loss, producing a drastic inhibition of seedling growth, with total loss of chlorophyll, which was observed at a concentration of 0.5 mM, already in the intermediate time point of sampling (Fig. 2, t-u) (Table 1, lines 10, 11 and 12).

Images from a to i correspond to controls, either with the standard MS culture medium alone (a to c), or with the addition of pyrophosphate (d to f), or with the addition of tripolyphosphate (g to i): the analysis of the features of these control samples (a to i) does not reveal significant differences among them. From j to l: culture in the presence of 0.1 mM etidronate for 4, 8 and 11 days, respectively; images j and k are practically unaltered with respect to controls; root length similar to controls, but secondary roots are much less developed (l). From m to o: culture in the presence of 0.05 mM clodronate for 4, 8 and 11 days, respectively; at the 4th day of growth, primary roots are shorter (m) than the controls (a, d and g) and the root growth appears practically arrested in successive samples (n to o); secondary roots are totally absent (o). From p to r: culture in the presence of 0.5 mM

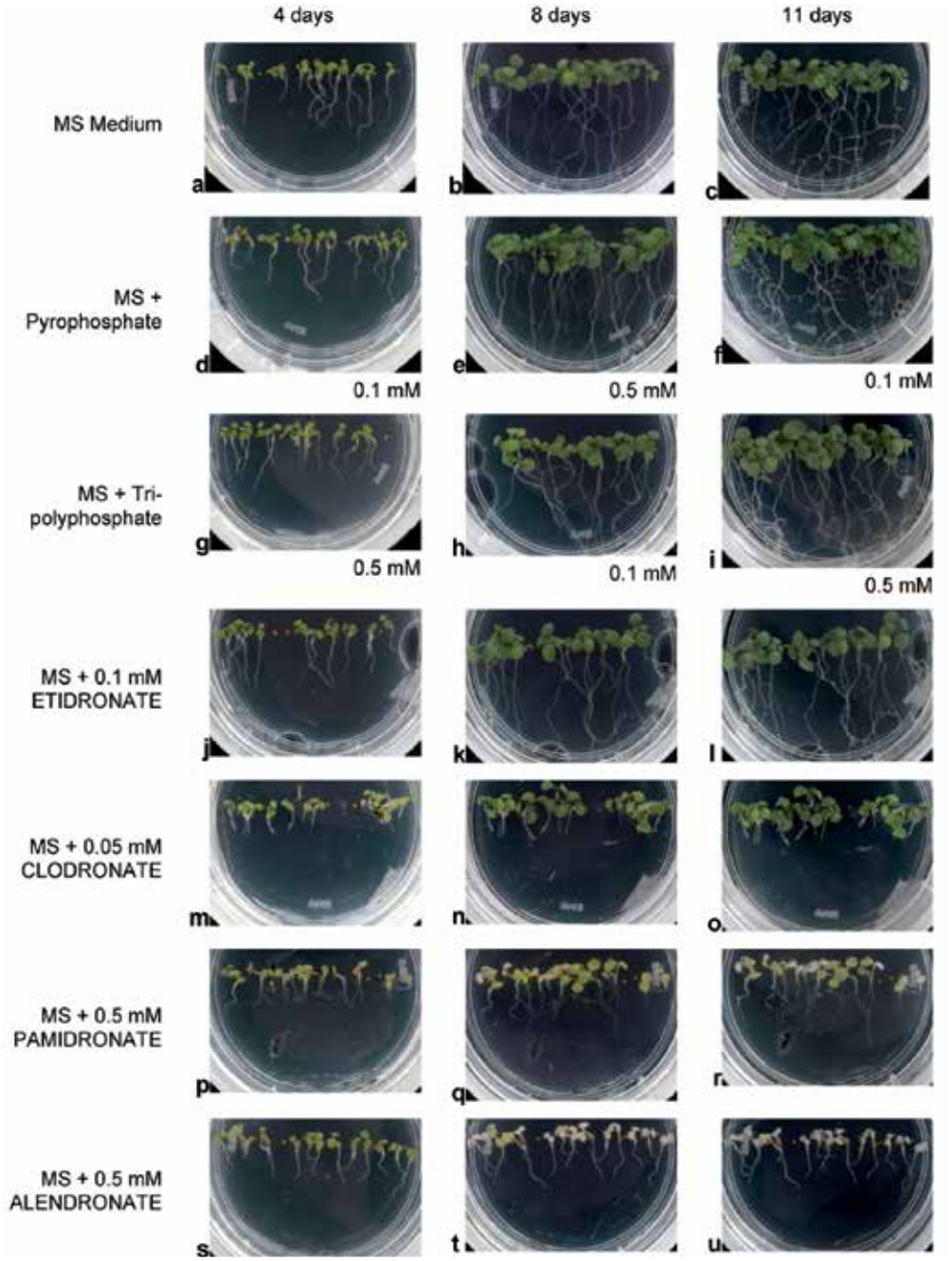


Fig. 2. Selected images of the results obtained after the germination and growth of *Arabidopsis thaliana* seedlings in the presence of four different bisphosphonates.

pamidronate for 4, 8 and 11 days, respectively; at the 4th day of growth, primary roots are shorter (p) than the controls (a, d, g); the root growth does not progress further (q to r); leaves show a pale yellowish colour in all cases, but this effect is more intense as the culture

in the presence of the drug progresses in time. From s to u: culture in the presence of 0.5 mM alendronate for 4, 8 and 11 days, respectively; the root growth is severely hampered in these conditions; 8 - and 11-day old seedlings show an almost white colour of leaves, indicative of a very serious loss of chlorophyll.

The above results can be interpreted as a consequence of the toxic effects of BPs on the mevalonate pathway of *Arabidopsis thaliana* (Fig. 1). Isoprenoids, the most diverse group of natural products, are synthesized in prokaryotes and eukaryotes, animals and plants by condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate. They play important and diverse roles in the synthesis of quinones, sterols, prenylation of proteins, photosynthetic pigments, hormones, attractants for pollinators in plants, and others. Certainly, most of the studies on the synthesis of isoprenoids were carried out in animal tissues; however, after the more recent discovery of the synthesis of isoprenoids by the mevalonate independent pathway operating in plants, new approaches on their metabolism and function have been undertaken in other biological model systems (Eisenreich et al., 2004; Lange et al., 2000; Rodriguez-Concepcion & Boronat, 2002; Rohmer, 1999). The plastid mevalonate independent pathway involves the synthesis of 1-deoxy-D.xilulose from pyruvate and glyceraldehydes-3-phosphate. There is a cross talk between the cytosolic and plastidial pathway for the synthesis of isoprenoids in *Arabidopsis thaliana* (Laule et al., 2003). In relation with the postulated inhibitory role of bisphosphonates on the synthesis of isoprenoids, it could be noted that the two last compounds in that synthesis by the mevalonate independent pathway (2-C-methyl-D-erythritol 2, 4, cyclo pyrophosphate and hydroxymethybutenyl-4 pyrophosphate) both contain a terminal pyrophosphate; in addition, isopentenyl and dimethylallyl pyrophosphate, common to the two pathways, also contain a terminal pyrophosphate (Fig. 1). All these points could be raised in favour of the inhibitory effects of bisphosphonates on the synthesis of isoprenoids and hence on the development of *Arabidopsis thaliana.*

The mevalonate pathway is responsible, in plants, of the production of different phytohormones, among which cytokinins (Letham & Palni, 1983). Cytokinins have been shown to reverse the effects of lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the enzyme catalyzing the synthesis of mevalonate (Crowell & Salaz 1992). Although root growth and lateral growth formation are preferentially determined by auxins, a role of cytokinins in root development has been described, coherent with their role in promoting cell division, specifically in root meristematic cells. Cytokinins, synthesized in the root cap, promote cytokinesis, vascular differentiation, root apical dominance and gravitropism (Aloni et al., 2006). Therefore, the observed effects of BPs on root growth and development may, in principle, be attributed to an inhibitory effect of BPs on cytokinins, although an indirect effect on the synthesis and/or transport of auxins cannot be excluded either. Whereas the differential effect of the various assayed BPs on root growth and development is unequivocal, additional work is necessary to understand the reasons for this differential effect and the mechanisms of the inhibition throughout the action of one or more phytohormones.

Finally, the importance of the mevalonate pathway in the biological systems can also be contemplated from an evolutionary viewpoint: isoprenoids are the oldest known biomolecules recovered from sediments, as old as 2.5 billion years (Summons et al., 2006); the mevalonate pathway is germane to archaebacteria (Lange et al., 2000) and finally, farnesyl diphosphate synthase and polypropenyl synthase activities have been reported as

present in the hypothetical Last Universal Common Ancestor (LUCA) (Ranea et al., 2006). All the above points could be also considered as examples of the multiple relationships interconnecting all the biological cycles on earth.

4. Conclusions

Bisphosphonates are widely used in the treatment of osteoporosis and hence their great importance in clinical investigation. Upon their administration they bind to the bone tissues where they exert a noxious effect mainly on the mevalonate pathway of the osteoclasts. Given the universality of this pathway, present in bacteria, plants and animals, it seemed to us of interest to analyze the effect of etidronate, clodronate (non containing nitrogen or non-N-BPs), pamidronate and alendronate (N-BPs) on some visible traits of the model plant *Arabidopsis thaliana*. The seeds were grown in a medium containing three concentrations (0.05 mM, 0.1 mM or 0.5 mM) of each bisphosphonate and the growth of primary and secondary roots and formation of chlorophyll were observed during early development of the seedlings. Each bisphosphonate showed a different pattern of influence on those parameters. In general, the inhibitory effects were, in increasing order: etidronate, pamidronate, clodronate, and alendronate. Specific effects on the evaluated parameters ranged from simple reduction in the number and length of secondary roots caused by 0.1 mM etidronate, only after 11 days of culture, until a drastic inhibition of seedling growth, with total loss of chlorophyll observed with 0.5 mM alendronate already in the intermediate time point of sampling. The utility of the use of plants to analyze the action of bisphosphonates is suggested.

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Osteoporosis is a public health issue worldwide. During the last few years, progress has been made concerning the knowledge of the pathophysiological mechanism of the disease. Sophisticated technologies have added important information in bone mineral density measurements and, additionally, geometrical and mechanical properties of bone. New bone indices have been developed from biochemical and hormonal measurements in order to investigate bone metabolism. Although it is clear that drugs are an essential element of the therapy, beyond medication there are other interventions in the management of the disease. Prevention of osteoporosis starts in young ages and continues during aging in order to prevent fractures associated with impaired quality of life, physical decline, mortality, and high cost for the health system. A number of different specialties are holding the scientific knowledge in osteoporosis. For this reason, we have collected papers from scientific departments all over the world for this book. The book includes up-to-date information about basics of bones, epidemiological data, diagnosis and assessment of osteoporosis, secondary osteoporosis, pediatric issues, prevention and treatment strategies, and research papers from osteoporotic fields.

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