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Adipose Tissue

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Meet the editor

Leszek Szablewski is a professor of Medical Sciences. He graduated from the University of Warsaw, Poland. He received his MS. degree from the Faculty of Biology, University of Warsaw, PhD degree from the Institute of Experimental Biology Polish Academy of Sciences, and Habilitation degree from the Medical University of Warsaw. He received the title of Professor from the President of Poland. Prof. Szablewski has experience in cell physiology, genetics, and pathophysiology. He works on damages of glucose transport into cells and disturbances in expression of glucose transporters due to diseases, surgical procedures and selected tumor cells. He is the Head of Chair and Department of General Biology and Parasitology, Medical University of Warsaw. He has given lectures, seminars and exercises for students of Medical University.

Contents

Preface

Adipose tissue, a specialized connective tissue, plays significant roles in humans as well as in animals. Adipocytes are the only cells that store lipids and triacylglycerol and synthesize fatty acids. This tissue also protects against cold and mechanical injury, is involved in the process of thermogenesis and secretes molecules that may have beneficial role and may be associated with pathologies and diseases. In the past several years, the knowledge of adipose tissue, especially as an endocrine organ, has been growing. This book aims to provide an overview on the topic of adipose tissue, its types, distribution in the body, role and so on. The authors discuss adipose tissue from different aspects to enhance the understanding of this tissue and its role in the human body.

This book mainly contains five sections. Section 1 that contains only one chapter presents the general characteristics of adipose tissue. Section 2 begins an overview in detail of particular types of adipose tissue, its functions, characteristics and so on. Section 3 describes the pathologies and diseases in which the adipose tissue is involved. The chapters included in this section describe, for example, obesity as a worldwide health problem. Section 4 presents adipose tissue as an endocrine organ. White adipose tissue is highly active metabolic tissue. Secreted substances, such as cholesterol, retinol, steroid hormones, prostaglandins and proteins known as "adipokines", influence human physiology and pathology. Authors describe the effects of these molecules. Finally, Section 5 focuses on the signaling system in adipose tissue. Intracellular signaling pathways are involved in the processes that occurr in cells and tissues. On the other hand, these processes determine the functions of these cells and tissues.

I would like to thank Ms. Lada Bozic for her great efforts in the book planning and editing during the process of book publication.

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Section 1

Introduction

Chapter 1

Introductory Chapter: Types of Adipose Tissue

Leszek Szablewski

Additional information is available at the end of the chapter

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1. Introduction

Brown adipose tissue (BAT) is found in fetuses and newborn. In adult humans, this type of adipose tissue is practically absent. The active brown adipose tissue in adult humans is present at discrete sites, such as in the upper trunk (in cervical, supraclavicular, paravertebral, pericardial, mediastinal, and mesenteric areas) [1]. In mammals, BAT is involved in process of thermogenesis. It produces heat metabolizing fatty acids. Its specific role is due to uniquely expressed in mitochondria of uncoupling protein 1 (UCP1). Activation of UCP1 stimulates uptake of lipids and glucose from circulation to process of thermogenesis. In women, as compared to men, functional brown adipocyte is more common. The mass of BAT depends on overweight, obesity, and age. In these people, the mass of brown adipose tissue is reduced.

White adipose tissue (WAT) store of lipids and during fasting, the release of fatty acids that in process of β-oxidation, is the source of adenosine triphosphate (ATP). ATP is necessary for all living organisms; for bacteria, fungi, plants, animals, and humans. WAT is also known as a major secretory organ and high active metabolic tissue. It secretes, for example, cholesterol, retinol, steroid hormones, prostaglandins, and proteins known as "adipokines." Some of these molecules may be associated with pathologies such as obesity, insulin resistance. These substances may increase the risk of metabolic syndrome, cardiovascular diseases, and others. As examples of substances synthesized and released by WAT are: Leptin, tumor necrosis factor-α, adiponectin, and interleukin-6.

Leptin is a peptide hormone synthesized and released mainly by adipose tissue. Many types of human organs express leptin, such as placenta, gastric fundus mucosa, and skeletal muscle, but subcutaneous adipocytes are responsible for 80% of total leptin production [2]. It is involved in the regulation of energy balance and food intake. Leptin plays also a role in reproduction. It was observed that hypothalamic hypogonadism in humans and in rodents is due to deficiencies or insensitivity to leptin. Other roles of leptin are increase of cytokine

production and macrophage adhesion and phagocytosis, modulation of blood pressure, influence on insulin sensitivity of peripheral, hepatic and skeletal muscle, and modulation of pancreatic β-cell function [3].

Tumor necrosis factor-α is a proinflammatory cytokine. It is synthesized mainly by macrophages and lymphocytes, but in humans also in low quantities in adipocytes. This cytokine influences on inflammation, apoptosis, cytotoxicity, synthesis of interleukin-1 and interleukin-6, and on adipocyte metabolism. It alters intracellular insulin signaling and induces insulin resistance. Its expression and secretion correlate with BMI increases in obesity and decreases in weight loss.

Adiponectin is highly expressed in adipocytes. Its expression depends on the distribution of adipose tissue in the human body. The levels of this protein are higher in subcutaneous adipose tissue than in visceral adipose tissue. It was observed the negative correlation between the degree of obesity and levels of adiponectin in circulation. In patients with type 2 diabetes, obese with insulin resistance, and in patients with coronary heart disease, adiponectin is not expressed [2].

Interleukin-6 is synthesized by many cell types and different tissues, including white adipose tissue. In the absence of an acute inflammatory process, WAT synthesizes substantial amounts of IL-6. It might represent about 15–30% of circulating levels [4]. Like adiponectin, its secretion depends on the distribution. In visceral adipose tissue, its secretion is three times higher as compared to subcutaneous adipose tissue. It was observed the link between IL-6 and obesity, inflammation, and coronary heart disease.

Adipose tissue synthesize and release also many others molecules: Resistin, retinol binding protein 4 (RBP4), vaspin, visfatin, omentin, chemerin, serum amyloid A (SAA), acylation stimulating protein (ASP), plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, vascular endothelial growth factor (VEGF), hepatocyte growth factor, transforming growth factor-B (TGF-B), insulin-like growth factor-1 (IGF-1), macrophage migration inhibitory factor (MIF), lipoprotein lipase, cholesterol ester transfer protein (CEPT), prostaglandins, estrogens, glucocorticoids, and apelin.

The beige adipose tissue (BeAT) is similar histologically to brown adipose tissue. Different stimuli, such as cold, exercise, thyroid hormones, bile acids, and cause differentiation of white adipose tissue into brown adipocytes. Browning of white adipose tissue is an adaptive and reversible response of WAT to stimuli. There are used different synonyms to describe the differentiation of WAT into BAT–browning, britening, and beiging. Beige (brite–from "brown in white") adipocytes have multilocular lipid droplets in the cytoplasm, numerous mitochondria as well as several intermediate features between WAT and BAT. The process of thermogenesis in BeAT, activated by cold, and may be mediated indirectly by the sympathetic nervous system. UCP-1 protein can be also involved in process of thermogenesis in BeAT.

In humans, there are two main sites of adipose tissue accumulation: Visceral and subcutaneous. Obesity is a worldwide health problem. It is defined as a body mass index (BMI) of ≥30 kg/m^2, and abdominal obesity is defined as waist circumference > 102 cm for men and > 88 cm for women. Obesity increases the risk of many diseases such as diabetes mellitus,

metabolic syndrome, cardiovascular diseases, cancers, and so on. Visceral and subcutaneous adipose tissues differ not only in their distribution. They express different genes involved in insulin resistance the pattern of expression of these genes is different, as well as these tissues differ in production and secretion of adipokines. It was observed that visceral abdominal obesity reduces the life expectancy of ~ 8 years. Central abdominal fat causes insulin resistance. It is suggested that this pathology is due to release of fatty acids from visceral depot into the portal vein, increasing gluconeogenesis, and hepatic glucose output. As mentioned above, visceral adipose tissue secretes adipokines that may cause lipotoxicity in peripheral tissues. On the other hand, in male patients with diabetes, visceral fat accumulation is less correlated with insulin resistance than subcutaneous fat accumulation.

This book aims to provide an overview of adipose tissue, its types, characteristics, role in humans, and animals. There are also described processes in adipose tissue involved in human health and diseases.

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Section 2

Characterization of Adipose Tissue

Chapter 2

Characterization and Differentiation of Adipose Tissue by Spectroscopic and Spectral Imaging Techniques

Fatma Küçük Baloğlu and Feride Severcan

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Abstract

Adipose tissue is a metabolically active endocrine organ having a distribution in a variety of locations in whole body; therefore, it is crucial to understand the adipocyte metabolism in health and disease. Spectroscopic techniques such as Fourier transform infrared (FTIR), Raman, nuclear magnetic resonance (NMR) are widely used to characterize biological systems by monitoring cellular molecules such as lipids, carbohydrates, and proteins. Obesity or insulin resistance-induced molecular alterations in adipose tissue can be detected using these techniques. Spectral imaging of adipose tissue provides high-quality information involving molecular compositional, structural, and functional alterations for characterization and differentiation of adipocytes (brown, white) in different adipose tissue regions (visceral, subcutaneous, etc.). In this chapter, applications of spectroscopic and spectral imaging techniques for characterization and differentiation of various adipose tissues will be discussed, which will shed light to better understand adipose tissue metabolism and provide new insight into diagnosis and treatment of some metabolic diseases such as obesity.

Keywords: adipose tissue, characterization, differentiation, spectroscopy, spectral imaging, infrared (IR), Raman, NMR, MRI, adipocyte, brown adipocyte, white adipocyte, visceral adipose tissue, subcutaneous adipose tissue, obesity, diabetes

1. Introduction

Adipose tissue is a complicated, crucial, and highly active metabolic and endocrine organ. Adipocytes are the cells that primarily constitute adipose tissue. Besides adipocytes, adipose tissue also includes the stromal vascular fraction (SVF) of cells including fibroblasts, vascular endothelial cells, and a variety of immune cells such as macrophages. Adipose tissue is the primary storage location for excess energy but it may also be defined as an endocrine organ. Adipose tissue

not only replies to afferent signals from endocrine system and the central nervous system but also releases many components having crucial endocrine functions. These components involve leptin, adiponectin, tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6), plasminogen activator inhibitor-1, proteins of the renin-angiotensin system, and resistin [1]. They are responsible for controlling of immune system, thermogenesis, and also neuroendocrine function [2].

Adipose cell, also called adipocyte or fat cell, is basically a connective tissue cell specialized in synthesize and storage large amounts of fat. Adipocytes are complex cells composing a number of signals including cytokines, hormones, and growth factors. These components can affect the neighboring cells and target tissues associated with energy metabolism, physiologic, and pathologic processes [3]. There are two types of adipocytes: white adipocytes contain large lipid droplets, a small amount of cytoplasm, and decentralized nucleus; while brown adipocytes contain lipid droplets of varied size, a large amount of cytoplasm, a lot of mitochondria, and round, centralized nucleus [4]. White adipocytes are globular cells whose changeable size principally depends on the size of the single lipid droplet accumulated within them. These lipid droplets are composed of triglycerides (TGs) and they take up more than 90% of the cell volume. White adipocytes account for storing of energetic molecules to provide energy to the cells between the meals. Brown adipocytes also include triglycerides as multiple small vacuoles; they have generally polygonal shape with a variable diameter. Mitochondria are the most characteristic organelles of brown adipocytes. The color of brown adipocytes proceeds from its high mitochondrial density and high vascularization [5, 6].

The adipose tissues are classified based on their colors because there are alterations in histological composition between white adipose tissue (WAT) and brown adipose tissue (BAT) [4]. Amounts of these tissues in the body show variation with respect to age, gender, strain, and environmental elements. The conventional function of WAT is to supply a long-term energy fuel stock which can be mobilized during lack of food by releasing of fatty acids for oxidation in related organs [7]. Moreover, WAT secretes a range of fundamental components affecting the metabolism of the whole system like leptin that can affect especially the eating behavior [8]. Leptin is a hormone of long-term regulation of energy balance, suppressing food intake and then inducing weight loss. BAT and WAT differ from each other in many respects. BAT has a completely different role than WAT, because it is responsible for thermogenesis. Since BAT requires more oxygen, it has more capillaries than WAT. Nerve supply is also more intense in BAT than in WAT. On the other hand, BAT can pass the energy gained from nourishment to energy [9, 10]. Uncoupling protein 1 (UCP1) that is specifically expressed in BAT is responsible for this conversion of the energy that is not utilized in oxidative metabolism [11]. Noradrenaline is capable to activate the beta-3-adrenoceptors that promotes brown adipocytes to form heat. The brown adipocytes can lose most of their brown characteristics, when they are not stimulated adrenergically, this process cause transdifferentiation of brown adipocytes into white adipocytes [4, 12]. This morphological transformation is reversible and it occurs with UCP-1 gene inhibition and leptin gene activation [13]. BAT phenotype in adipose tissues is so crucial in rodents for the inhibition of various metabolic diseases such as obesity and diabetes [4].

Adipose tissue is approved as a crucial, complicated, and metabolically active endocrine organ having a distribution in a variety of locations in whole body differently from the other

organs [1, 14]. The murine adipose organ composed of two main subcutaneous storages (anterior and posterior) and several visceral storages (mediastinal, omental, mesenteric, perirenal, retroperitoneal, perigonadal, and perivesical) [6]. The locational fat distribution is more critical than whole fat content in the body in the matter of obesity-linked metabolic diseases [15]. It has been reported that more than 80% of total body fat is constituted from subcutaneous adipose tissue (SCAT) in the body and approximately 10–20% from visceral adipose tissue (VAT) in adults [16]. SCAT consists of two different anatomical layers where superficial and deep layers are separated by the fascia superficialis (Scarpa's facia). Subcutaneous fat depots represent 80% of the whole fat mass in normal weight subjects. VAT is an intraperitoneal adipose tissue and it primarily consists of the omental and mesenteric fat depots [17]. SCAT and VAT differ from each other in respect to the type of adipocytes, lipolysis process, endocrine functions and their reaction to insulin and other hormones [18]. VAT has a crucial role in the expression of inflammatory cytokines and secretion of various hormones causing the metabolic effects of obesity since it possesses a special position near portal vein [15, 18–21]. VAT is also different from the SCAT due to the fact that visceral adipocytes have more lipolytic activity metabolically and they are more active than subcutaneous adipocytes [22, 23]. VAT involves greater number of large adipocytes contrary to SCAT, which contains the small adipocytes. These small adipocytes are more insulin-sensitive and more prone to free fatty acid (FFA) and triglyceride (TG) uptake in order to avoid their storage in non-adipose tissue [24, 25]. On the other hand, VAT may have a role in lipolysis of central SCAT causing the release of peripheral FFA [20]. VAT is more sensitive to the catecholamine-induced lipolysis and less sensitive to the antilipolysis action of insulin; therefore, VAT possesses a higher glucose uptake upon insulin stimulation; thus, it becomes more insulin resistant than SCAT [16, 26, 27]. VAT has more vascular structure which is rich in blood supply and more nerve cells than SCAT and they also differ in capacity to produce and secrete adipokines [18].

Obesity results from a chronic imbalance between the level of energy intake and consumption causing extreme weight gain. Obesity constitutively results in storage of triglycerides in different adipose tissues [28]. The increase in fat mass results in the greater adipocyte size (hypertrophy) and increased numbers of adipocytes (hyperplasia). The hypertrophy, hyperplasia, or both of them occur in return for energy imbalance that may alter the location of the adipose tissue [29]. The VAT deposition occurs only if SCAT capacity has been reached to the maximum in early stage of obesity [30]. These changes related to adipocyte hypertrophy could be the first steps toward adipocyte dysfunction. Obesity can be defined as the expansion of VAT and SCAT mass in the body, causing alterations in cellular biology, that is, disturbed glucose and lipid metabolism. Though, abdominal obesity is determined by the storage of both VAT and SCAT in the body, VAT is considered as having more critical role in the metabolism of obesity [20].

Recent studies point to the importance of adipose tissue in diagnosis and treatment of obesity and obesity-related diseases. In current chapter, after mentioning briefly about adipose tissue, the applications of mainly infrared (mid and near), Raman, and nuclear magnetic resonance spectroscopic and microspectroscopic techniques in characterization and differentiation of adipose tissues will be discussed in detail. The other spectroscopic techniques such as circular dichroism, electron spin resonance and fluorescence spectroscopy were not included, because their application to adipose tissues are very limited or none so far.

2. Characterization and differentiation of adipose tissue by vibrational spectroscopic and spectral imaging techniques

2.1. Basis of vibrational spectroscopy

Spectroscopy is the study of the interaction of electromagnetic radiation in its all forms with matter which gives a data called spectrum. "A spectrum is a plot of the intensity of energy detected versus the wavelength, wavenumber or frequency of the energy" [31]. Electromagnetic radiation is the main source of energy used for spectroscopic studies. These spectroscopic studies include irradiation of a sample with several forms of electromagnetic radiation. Monitoring of the spectral parameters derived from absorption, emission, or scattering of the electromagnetic radiation as a result of interaction with matter provides information about the atomic and molecular design of samples [32].

The electromagnetic radiations covering a very wide range of wavelengths have been utilized by several techniques that are used in both biology and medicine. Among these techniques, infrared (IR) spectroscopy has a potential as a powerful tool for structural characterization of molecules and it was accepted as a crucial tool for understanding the structure of biomolecules. Besides IR spectroscopy, another vibrational spectroscopic method that is in current use includes Raman spectroscopy. The main principle based on these physical techniques is the transitions between quantized vibrational energy states of molecules as a result of absorption of electromagnetic energy. In IR spectroscopy, the incident electromagnetic radiation matches the difference between the transition energy levels and this process occurs with high probability. This energy is in the 14,000–4000 cm^{-1} range for the near infrared (NIR), and in the 4000–400 cm^{-1} range for mid-infrared spectroscopy [33].

In addition to absorbance of light, the sample molecules can also scatter light and this can be detected at 90° to the propagation direction of the incident beam. This phenomenon known as Raman scattering is the basis of Raman spectroscopy. Although incident and scattered energy are in the visible range of electromagnetic radiation, the transition between the vibrational energy levels is induced. This is a low-probability event, since the incident energy does not match the energy differences between the vibrational energy levels.

Since each molecule will have its own unique vibrational characteristics, each molecule possesses a unique infrared or Raman spectrum. This fact makes vibrational spectroscopy a golden tool in the characterization of molecular structure. Some properties of vibrational band, such as its position, intensity/area, and width, can be utilized for monitoring a certain functional group or molecule in different conditions. These conditions can be metabolic conditions, alterations in the environmental factors, or genetic modifications in the organisms which are able to alter the molecular composition, concentration, structure, and function of biomolecules. Since these alterations can affect the vibrational transitions which are directly reflected in the vibrational spectral bands and therefore, they can be evaluated by using vibrational spectroscopy techniques. Vibrational spectroscopy obtains qualitative and quantitative information as a rapid, accurate, cost-effective, and operator-independent technique for identification of the spectral differences arising from pathological or environmental conditions [33].

Fourier transform infrared (FTIR) and Raman spectroscopy are complementary techniques for the study of molecular vibrations and structure. The combination with a microscope results in an analytical method known as microspectroscopy that allows spatially resolved investigation of the biochemical compounds of biological samples. The high spatial resolution makes it possible to study areas down to approximately 10 × 10 μm with FT-IR microspectroscopy and approximately 1 × 1 μm with Raman microspectroscopy [34]. As the most common techniques of vibrational microspectroscopy, infrared and Raman microspectroscopy allow determination of the inherent vibrational spectra of the biochemical components of a cell [35]. Hence, they allow visualization of cellular composition based on their chemical properties and provide metabolic clues in disease diagnosis and therapy. Therefore, these techniques have been approved widely in the field of biology and medicine because of their fast and non-invasive nature [36].

Application of spectroscopic and spectral imaging techniques has been gaining importance in discrimination and characterization of different tissue types such as adipose tissue. Research in this field has focused on the characterization of differentiation of adipose tissue by investigation of molecular composition, structure, and distribution of adipocytes to better understanding of roles of different adipocytes in metabolism in healthy and disease states such as obesity and type II diabetes. Another application of this field is on obesity and diabetes-induced alterations in adipose tissue in order to determine spectral parameters that can be used in the generation of new and rapid methods for diagnosis and treatment of these diseases in the future. In the current chapter, various applications of spectroscopic and spectral imaging techniques in the evaluation of different adipose tissue types will be presented.

2.2. Applications of vibrational spectroscopic and imaging techniques on adipose tissue

Biological samples contain biochemical substances such as carbohydrates, proteins, lipids, and nucleic acids, and these biochemical molecules have their unique vibrational fingerprints individually. In biological systems, the infrared spectrum is complex and the sum of contributions coming from proteins, lipids, nucleic acids, and other chemical species present in the cells [37, 38]. **Figure 1** shows the representative spectra of adipose tissue of control and obese mice in the 4000–650 cm^{-1} region and the main bands are labeled in the figure, and band assignments are given in integrated table.

FTIR spectroscopy, as a sensitive technique detecting the alterations in the functional groups of biological tissue constituents, is capable to differentiate the spectra of healthy and diseased biological samples [45–48]. These disease conditions induce significant changes in molecular content, concentration, structure, and dynamics in tissues, cells, body fluids, and membranes. These alterations can be detected rapidly and sensitively without using external agents by attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy [49, 50]. Sen et al. [51] aimed to investigate the effects of obesity on macromolecular alterations in order to characterize berlin fat mice (BFMI) lines according to the macromolecular alterations within SCAT and VAT by using ATR-FTIR spectroscopy. For this purpose, detailed spectral analysis was performed to characterize lipid and glycogen content of adipose tissues of mouse models

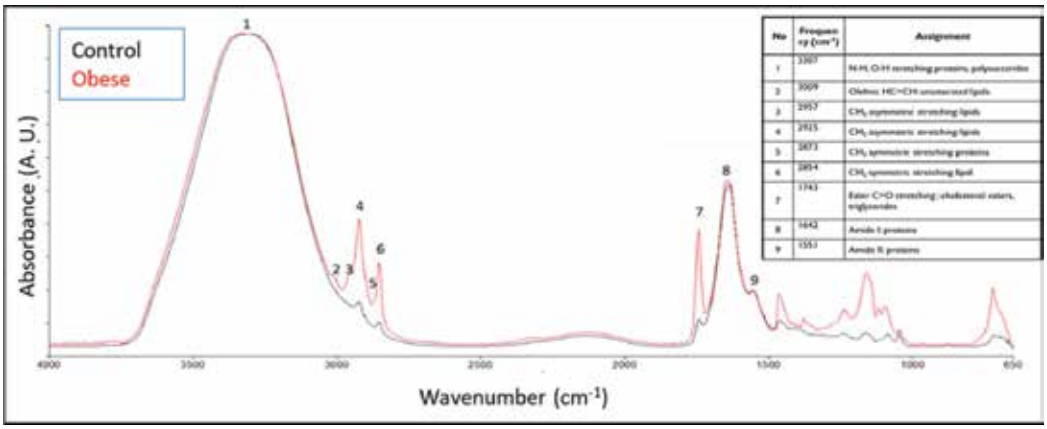

Figure 1. The representative ATR-FTIR spectra of adipose tissue of control and obese mice in 4000–650 cm^{-1} region. The spectra are normalized with respect to amide a band located around 3300 cm^{-1}. General band assignment of FTIR spectrum of adipose tissue based on literature was given in box [39–44].

of spontaneous obesity. This study revealed that there was a loss of unsaturation in BFMI860 and 861 lines in SCAT with a decrease in the hydrocarbon chain length of lipids suggesting an increased lipid peroxidation. In addition, there was an increase in saturated lipid and triglyceride content in all tissues of BFMI lines. These results demonstrated that SCAT also indicated considerable obesity-induced alterations. In conclusion, these results revealed alterations in lipid structure and content of BFMI lines, which may arise from different insulin-sensitivity levels of the lines. These obesity-induced alterations revealed by FT-IR spectroscopy in lipids of the lines, such as acyl chain length and degree of unsaturation, may be a consequence of the alterations in insulin sensitivity levels. Another important result of this study is that SCAT and VAT indicated significant obesity-induced alterations and both of them take a role in lipid metabolism in obesity. Furthermore, the current study clearly revealed the characterization power of ATR-FTIR spectroscopy in the precise determination of spectral variations in different adipose tissue components of mouse models of juvenile obesity without a high-fat diet induction.

Since each sample has a characteristic composition of molecules, IR spectroscopy provides a phenotypic fingerprint. This fact makes FTIR spectroscopy as a complementary technique for genomic approaches to detect unique genetic variants among individuals. In a recent study, ATR-FTIR spectroscopy was combined with a genetic approach to identify genetic differences responsible for phenotypic alterations in adipose tissue. The phenotypic characterization obtained by ATR-FTIR spectroscopic data was used to identify novel chromosomal regions contributing to distinct features of high-fat diet-induced obesity. The analytical technique of ATR-FTIR spectroscopy accompanied with quantitative trait loci (QTL) analysis was introduced as a novel phenotyping method that enables to characterize the macromolecular composition of adipose tissue. By performing this method, the alterations between the BXD RI strains with respect to the trait of interest reflecting genetic variation were obtained and two genomic regions that may have a function in obesity-induced tissue dysfunction were revealed [52].

Chemometrics can be defined as the application of statistical and mathematical calculations to obtain the information from the vibrational spectra [53]. Since vibrational spectra have many

molecular-based specific spectral peaks [54]. Multivariate statistics is convenient in spectral analysis because it improves the simultaneous inference of multiple spectral intensities, which enhances the precision and predictive ability of the analysis [55]. When coupled with appropriate multivariate statistical methods, FTIR spectroscopy can be a discriminatory technique with specific spectral markers [56]. Different multivariate analysis methods coupled with FTIR spectroscopy were successfully applied to the diagnosis of several diseases, such as obesity, diabetes, cancer, Alzheimer's disease [46, 47, 57–59]. In an obesity study, triglyceride band located at 1770–1720 cm^{-1} spectral region was proposed as a more sensitive obesity-related biomarker using the diagnostic potential of FTIR spectroscopy coupled with multivariate analysis in SCAT and VAT [57]. Principal component analysis (PCA) firstly was performed to examine the possible clustering of samples and to determine IR spectral bands that can differentiate the control and obese adipose tissue samples. The PCA results showed that the most dramatic difference in loading plots of control and obese groups was obtained from the 1770–1720 cm^{-1} region which belongs to triglyceride band as shown in the **Figure 2A** and **B**. This result implied that the triglyceride region has a significant contribution in the discrimination of the control and obese groups for both types

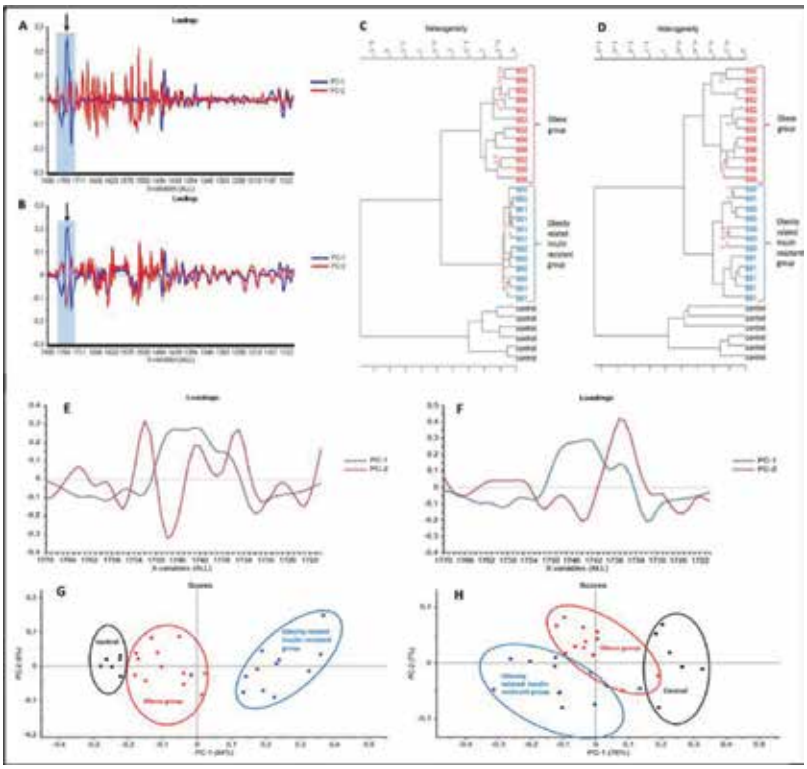

Figure 2. PCA loading plots for VAT (A) and SCAT (B) of control and obese groups in the 1800–1000 cm^{-1} spectral region. The triglyceride region is shown by arrow. Hierarchical clustering of control, obese, and obesity-related insulin resistant groups in SCAT (C) and VAT (D) in the 1770–1720 cm^{-1} spectral region. Control group (black), obese group (BFMI 852–856) (red), obesity-related insulin resistant group (BFMI 860–861) (blue). PCA loading plots of control, obese, and obesity-related insulin-resistant groups for SCAT (E) and VAT (F) samples in the 1770–1720 cm^{-1} spectral region. PC1 versus PC2 scores plot of the second derivative vector normalized spectra in the same range of SCAT (G) and VAT (H). Control group (black), obese group (BFMI 852–856) (red), obesity-related insulin-resistant group (BFMI 860–861) (blue) (Reproduced from [57], with permission from John Wiley and Sons).

of adipose tissues. Consistently, in hierarchical cluster analysis (HCA) results, a successful differentiation between the control, obese, and obesity-related insulin resistant groups was obtained in the triglyceride region with 100% sensitivity and specificity values as demonstrated in **Figure 2C** and **D**. In addition, PCA loading plots indicated a high difference between PC1 and PC2 which strongly indicates a successful differentiation in this region (**Figure 2E** and **F**). The PC1 versus PC2 scores plot for the triglyceride spectral range, as shown in **Figure 2G** and **H**, also supported the existence of a successful discrimination between the control, obese, and obesity-related insulin-resistant groups in both types of adipose tissues. Another finding is reported that the effects of obesity on the VAT highly correlate with the SCAT. Based on these results, this diagnostic technique can be transferred to medical research in the field of obesity. Since the SCAT is more accessible than the VAT for medical interventions, SCAT can be used in biopsies and bariatric operations preferably. The discriminatory power of FTIR spectroscopy coupled with multivariate analysis in diagnosis of obesity can be easily examined in human studies and this combined technique will shed light on the internal diagnosis of obesity in medical research [57].

FTIR microspectroscopy enables to acquire visible images of the investigated tissue whose each pixel consists of a spectrum arising from vibrational fingerprints. FTIR spectroscopic imaging is a label-free and nondestructive technique that quantifies the distribution of biologically relevant components in samples, concurrently revealing biochemical composition and morphology. FTIR imaging permits detecting the inherent vibrational mid-IR spectra of the biochemical constituents of cells and characterization of localized biochemical changes. The representative FTIR spectral maps obtained from control and obese groups of VAT samples are presented in **Figure 3**. Kucuk Baloglu et al. [60] aimed to characterize and compare VAT and SCAT according to biomolecular content and identify the possible transdifferentiation from brown to white adipocytes by using FTIR microspectroscopy and uncoupling protein 1 (UCP1) immunohistological staining in VAT and SCAT of spontaneously obese mice. In obese groups, a significant increase in the lipid/protein ratio, accompanied with a decrease

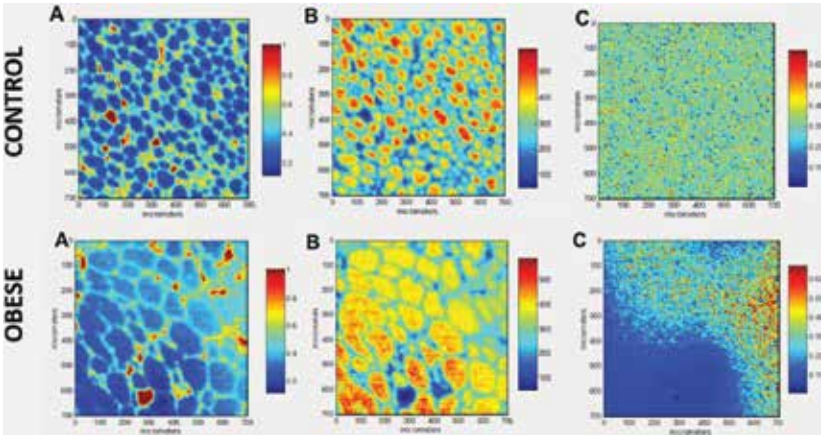

Figure 3. Representative FTIR spectral maps of VAT samples of control and obese mice. The absorbance in the spectral maps was represented in color-coded images, where low absorption was represented in blue and high absorption was represented in red color. [(A) amide I, (B) lipid to protein ratio, (C) unsaturation ratio].

of UCP1 protein content was obtained which might be arising from transdifferentiation of brown adipocytes to white adipocytes. In addition, when compared to control group, obese groups indicated a decreased unsaturation ratio, qualitatively longer hydrocarbon acyl chain length of lipids and increased amount of triglycerides revealed by FTIR microspectroscopy in both types of adipose tissues. Another finding indicated that SCAT was more prone to obesity-induced structural changes than VAT, which could originate from it, possessing a lower amount of brown adipose tissue. The current study clearly revealed the power of FTIR microspectroscopy in the precise determination of obesity-induced structural and functional changes in SCAT and VAT.

Aboualizadeh et al. [61] used FTIR microspectroscopy to characterize BAT and subcutaneous-WAT (s-WAT) derived from mice exposed to 30°C (thermoneutral condition), 24°C (room temperature), and 10°C (cold exposed). As seen from **Figure 4**, bright-field images of 10°C BAT (i) and s-WAT (ii) and some tissue regions with different morphological appearances are highlighted (red boxes). PCA results from the spectra that were derived from FTIR microspectroscopy facilitate the identification of spectra and enable to determine which peaks contribute the most in distinguishing the spectra. The loading plot from PCA shows that three positive bands attributed to proteins (3290, 1654, and 1544 cm^{-1}) and three negative bands attributed to C=O ester in phospholipids (1745 cm^{-1}), and symmetric and asymmetric stretching of CH_2 (2923, 2854 cm^{-1}). Scores in red color were attributed to the regions similar to the superimposed red boxes in **Figure 4B** and the green scores were attributed to the regions similar to the

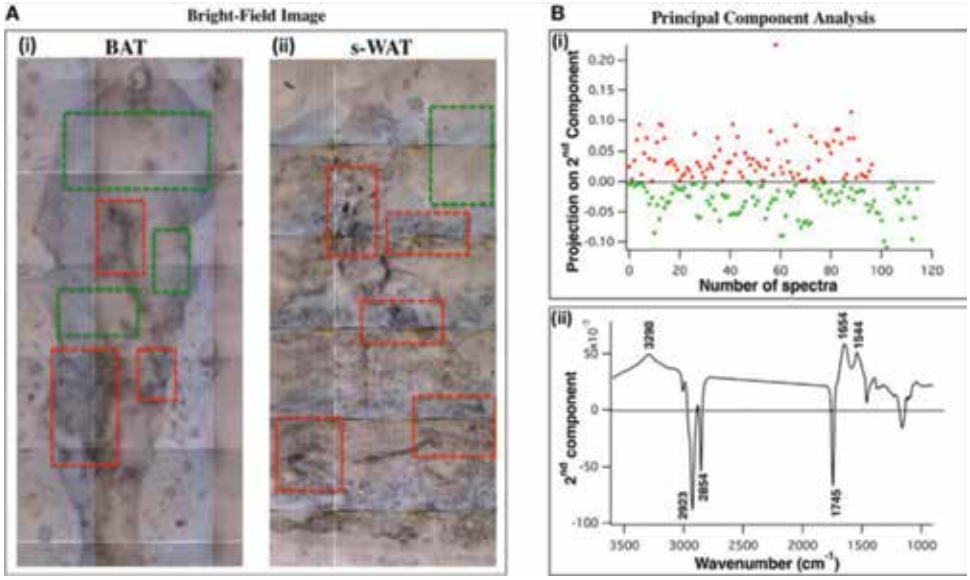

Figure 4. (A) Bright-field image of a cold-exposed BAT [(A), i] and s-WAT [(A), ii] are shown red boxes show the representative regions with different morphological appearances within tissue section and green boxes show more homogeneous regions within tissue. (B) Projection of spectra on the second component (scores) from PCA [(B), (i)] and associated loading plot [(B), (ii)] with the greatest varying wavenumbers are shown. Along PC2, positive scores are shown in red and negative scores are shown in green [(B)] (Reproduced from [61], with permission from Frontiers).

green boxes in **Figure 4B**. Spectral maps were generated by integrating over different spectral regions including signals of carbohydrates, proteins, and lipids. According the results, for 30, 24, and 10°C BAT and s-WAT showed gradual increases in protein to lipid ratio, as going from 30 to 24 to 10°C groups. Protein to lipid ratio was significantly higher in cold-exposed BAT and s-WAT compared to 24, and 30°C tissues. In addition, olefinic to lipid ratio indicated an increase in a progressive manner from 30 to 24 to 10°C groups. Cold-exposed tissues (10°C) showed a significantly higher level of olefinic to lipid ratio in BAT and s-WAT. FTIR microspectroscopy suggested that cold-exposed tissues AT had greater unsaturated lipid content than the warmer temperatures, and 1H nuclear magnetic resonance (NMR) studies validated these results as a complementary method which will be discussed in the following sections.

The near-infrared radiation (NIR) window, also known as the "therapeutic window," is the range of wavelengths that has the maximum depth of penetration in tissue. Since NIR is minimally absorbed by water and hemoglobin, making it a golden tool for medical applications, including functional analysis of biological tissues, as well as an analytical tool for diagnosing diseases [62]. Since BAT has abundant capillaries and mitochondria compared with WAT, near-infrared time-resolved spectroscopy (NIRTRS) is able to detect and evaluate the activation of BAT. When NIRTRS was used to assess the optical characteristics of the supraclavicular (SCV) BAT, it evaluated BAT density as a simple and noninvasive method by measuring the markers of tissue hemoglobin concentration and mitochondrial density [63, 64]. Moreover, NIRS as a noninvasive technique was combined with infrared thermography (IRT) to identify and monitor thermogenesis related to human BAT in adults with different BMI. In this study, lean and overweight subjects showed a consistent and highly localized increase in local temperature within the supraclavicular (SCV) region induced by a glucose ingestion followed by a cold stimulus in thermoneutral conditions (20°C). During OGTT and after cold stimulation, skin temperature was consistently higher in lean subjects compared to obese ones [65].

Raman spectroscopy is also widely used in biological applications because of its obvious advantages such as, being not influenced by water bands, high spatial resolution, high sensitivity to low-frequency vibrations, having less sample preparation steps [33]. Raman spectroscopy is especially suitable to *in vivo* measurements because the powers and excitation wavelengths used are non-destructive to biological tissues [66]. Raman spectroscopy is a proper method to monitor adipose tissue because this method is particularly sensitive to adipose tissue and lipids that exhibit large Raman scattering cross sections in comparison to other biological molecules. In a recent study, it has been demonstrated that Raman spectroscopy enables to detect WAT inflammation with high sensitivities and specificities in both mouse models of obesity and human tissues [67]. Beattie et al. [68] used Raman spectroscopy coupled with multivariate analysis to classify adipose tissue from four different species (chicken, beef, lamb, and pork). They reported that Raman spectroscopy with multivariate and neural network analytical methods allows to classify different species of an adipose tissue sample with higher than 99% accuracy. It is known that FT-IR and Raman spectroscopy applications have been used to examine different components in fats and oils including, among others, determination of trans-unsaturation [69, 70]. Olsen et al. [71] revealed that FTIR and Raman spectroscopy can be used as rapid, nondestructive method to determine omega-6 and omega-3 fatty acids in melted adipose tissue samples of porks. They also achieved to measure polyunsaturated, monounsaturated, and saturated fatty acids quantitatively in pork adipose tissue with nondestructive Raman spectroscopy [72].

3. Characterization and differentiation of adipose tissue by nuclear magnetic resonance spectroscopic and spectral imaging techniques

3.1. Basis of nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) is a spectroscopic technique that is used to monitor transitions between the energy levels due to nuclear-spin reorientation in an applied magnetic field. The incident energy which matches these energy differences is in the radiowave energy range. NMR spectroscopy and magnetic resonance imaging (MRI) can be used to examine a wide range of biological processes in systems from a single cell to a sample of tissue. One of the greatest advantages of NMR techniques is noninvasiveness. Hence, both biochemical (spectroscopy) and spatial information (imaging) can be obtained without destroying the sample. Another advantage of NMR methods is the lack of ionizing radiation for both imaging and spectroscopy. Most of non-NMR-based techniques use ionizing radiation in different forms for imaging or for in vivo studies. These two important advantages make NMR methods more useful for in vivo studies. Moreover, NMR spectroscopy and MRI can be combined to obtain metabolic, physiological, and anatomical data in a single experiment [73]. Other advantages of NMR can be listed as follows: It provides to study biological systems in their native aqueous environment; the NMR signals are sensitive to environment; the theory behind of it is well understood and therefore the relationship between spectral parameters and the information of interest (such as, concentration, structure, and dynamics) is defined well [74]. The main disadvantage of NMR spectroscopy is the lack of sensitivity due to very small population differences between the energy levels.

3.2. Applications of nuclear magnetic resonance spectroscopic and imaging techniques on adipose tissue

NMR spectroscopy, also known as magnetic resonance spectroscopy (MRS), is a non-invasive, ionizing-radiation-free analytical technique that that can be used to complement the more common MRI in the characterization of tissues in pathological conditions. Since MRI is able to distinguish between fat and water protons as regards their different magnetic resonance properties, adipose tissue samples can be characterized by this special contrast which is a phenomenon known as chemical shift. The noninvasive MRS and MRI techniques with various signal contrasts and underlying mechanisms allow to differentiate BAT from WAT morphologically and to assess BAT functionally because of its ability to enhance energy consumption through increased thermogenesis [75, 76]. Morphological distinction between BAT and WAT was obtained in rat by Osculati et al. [77] and they reported that there was a higher water content in BAT using 1H spectroscopy and suggested differences in fat-to-water ratio between BAT and WAT contributes to differences in signal intensities in MRI data. In addition, dimensions of BAT deposits can be determined by a combination of MRI and morphometry, and also MRI enables to differentiate areas of BAT responsive to acute adrenergic stimulation by giving information on the thermogenetically active tissue in vivo [78]. Verma et al. [79] characterized the biophysical properties of BAT and WAT by using quantitative translational diffusion measurements by high-resolution diffusion NMR spectroscopy to study the apparent diffusion coefficient (ADC) of fat molecules in rat BAT and WAT samples. The ADC of fat in BAT and WAT from chow diet rats was compared to high-fat diet rats to determine how the diffusion properties change based on obesity-related parameters such as lipid droplet size,

fatty acid chain length, and saturation. They reported that feeding with high-fat diet causes increased saturation, increased chain lengths, and reduced ADC of fat in WAT. Since, diffusion of fat was limited in BAT because of the presence of small lipid droplets, the ADC of fat was lower in BAT compared to WAT in rats fed both chow and high-fat diets. These findings indicated that in vivo diffusion could be a potential way for better characterization of BAT and WAT with metabolic alterations in both lean and obese sample. In both preclinical and clinical metabolic research, methods for in vivo investigation of adipose tissue would be invaluable for evaluating of metabolic diseases such as obesity. Branca et al. [80] proposed an in vivo NMR spectroscopic method to evaluate the effects of diet on fatty acid composition of the predominant chemical components of adipocytes in mice. The high resolution and sensitivity of the this method may be useful for the rapid detection of small changes in the composition of fatty acids in response to diet, exercise, and fat-metabolic diseases. Strobel et al. [81] achieved in vivo measurement of lipid composition in very small voxels (1.5 × 1.5 × 1.5 mm) in adipose tissue by using proton magnetic resonance spectroscopy (^{1}H-MRS) method in mice. This method uses localized point-resolved spectroscopy to collect ^{1}H spectra from voxels in intra-abdominal WAT and BAT depots for the characterization and differentiation of adipose tissue in rodent models of disease. This potential tool enables to study lipid metabolism in small animal models of disease during the initiation, progression, and manifestation of obesity-related disorders in vivo. In another MRI study, VAT and SCAT volume were measured and it was reported that children with an muscle fat content (MFC) ≥5%, compared with children with an MFC <5%, had a higher BMI and a higher VAT. On the other hand, there was no significant difference in SCAT, SCAT/VAT ratio [82].

4. Conclusion

Although spectroscopic techniques are in competition with electrochemistry for analytes detection in clinical chemistry, their some specific properties as in vivo analysis for metabolic studies and continuous monitoring, analysis of samples without reagents, detection of lower concentrations of biological components, capacity of diagnosis of diseases at very early stage, made the spectroscopic techniques state-of-the-art technology. Therefore, these techniques have taken part in the field of biomedicine especially in disease diagnosis and treatment-oriented monitoring in clinical investigations. The requirement of a rapid and operator-independent diagnostic method to characterize and differentiate cells and tissues is increasing with the expansion knowledge about metabolic diseases. With this diagnostic approach, they can be coupled with multivariate analysis to distinguish between normal and abnormal conditions in biological systems with a very high specificity and sensitivity.

In recent years, the evaluation of BAT using spectroscopic techniques gained momentum after BAT have been proposed as a potential therapeutic target for obesity and related metabolic diseases. Since targeting BAT, thermogenesis and monitoring BAT metabolism have a possible therapeutic potential for these metabolic diseases, the spectroscopic techniques focused on adipose tissue for medical investigations. Recent innovations in spectroscopy and microspectroscopy contributed to evaluation, characterization, and differentiation of the adipose

tissue. With this continuously evolving spectroscopic approach, these pioneer studies can be transferred to medical applications and they will shed light on the diagnosis and treatment of obesity and related metabolic diseases.

Conflicts of interest

The authors report no financial conflicts of interest. The authors are only responsible for the content and writing of this chapter.

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Chapter 3

Sirtuins in Adipose Tissue Metabolism

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Additional information is available at the end of the chapter

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Abstract

Obesity, a complex metabolic disorder linked to the development of several diseases, is characterized by both hypertrophy and hyperplasia of adipocytes. While white adipose tissue (WAT) is an energy storage site, brown adipose tissue (BAT) activation generates heat from nutrients by non-shivering thermogenesis. The human orthologue of silencing information regulator 2 (Sir2) which was recognized as a regulator of life span in *S. cerevisiae*, includes seven sirtuins which are NAD^+-dependent protein deacetylases distributed in different subcellular compartments. Sirtuins, particularly Sirt1, have emerged as important nutrient sensors and regulators of metabolism. Sirt1 has been shown to play a role in retarding the expansion of WAT while stimulating both differentiation and activation of brown adipose tissue as well as browning of WAT. This chapter focuses on the role of sirtuins in adipose tissue biology, their implications in obesity and potential as therapeutic targets.

Keywords: sirtuins, white adipose tissue, brown adipose tissue, adipogenesis, metabolic control, obesity

1. Introduction

Adipose tissue is a functionally diverse organ with remarkable plasticity to adapt to changes in energy balance and contribute to systemic regulation of metabolism. It is capable of expanding in response to over-nutrition preventing ectopic fat deposition in non-adipose tissues, as well as mobilizing stored lipids during starvation or energy demand. Apart from its storage function, its ability to produce multiple adipokines as an endocrine organ, influences functions of other metabolic tissues. The ability of adipose tissue to respond to changes in energy balance is finely regulated by molecular mechanisms linked to nutrient response and

the redox status of the tissue. Sirtuins are a group of NAD^+-dependent protein-deacetylating enzymes that play a key role in metabolic homeostasis. They influence genome stability, transcription and activity of several enzymes contributing to epigenetic regulation and act as key nutrient sensors.

Acetylation and deacetylation of histones and other DNA associated proteins are key epigenetic processes that can influence chromatin structure regulating the transition between highly condensed and transcriptionally less active heterochromatin state to loose and transcriptionally active euchromatin structure [1]. The extent of acetylation of histones depends, on one hand, on the balance between the activity of acetyl transferases such as histone acetyl transferase and deacetylases such as histone deacetylases (HDACs) [2, 3] and on the other hand on acetyl CoA which is an important metabolite at the junction of several metabolic pathways. Phylogenetically, eukaryotic HDACs are ancient proteins comprising of two subfamilies of proteins with different structure and function [4, 5]. While the enzymes belonging to the classical HDAC family are generally Zn-dependent enzymes which remove acetyl group from lysine residues on acetylated protein substrates with the addition of a water molecule, those belonging to the sirtuin family remove acetyl moieties bound to protein substrates to another substrate viz. NAD^+ cleaving it to nicotinamide and *O*-acetyl ADP–ribose. So far 11 members of the classical HDAC enzymes, subdivided into three classes (HDAC I, III and IV), and 7 members of sirtuins belonging to HDAC class III family have been identified.

Sirtuins, particularly SIRT1, play a key role in adipogenesis of white adipose tissue (WAT) and browning of WAT, metabolism of glucose and fat, insulin sensitivity, control of inflammation and energy homeostasis. Dysregulation of these physiological processes have major implications in the development of obesity and related metabolic diseases. The role of sirtuins in the regulation of adipose tissue biology and its implications in the development of obesity form the subject matter of this chapter. A number of reviews on the structure and functions of various sirtuins and their implications in aging and several pathological conditions are available [6–11].

2. Sirtuins in adipose tissue biology

2.1. Biochemistry and molecular biology of sirtuins

2.1.1. Classification and tissue distribution

Interest in understanding the role of sirtuins in mammalian system was driven by findings in calorie-restricted conditions in model organisms. The discovery of a protein in yeast, referred to as yeast-silencing information regulator-2 (Sir2) [12] followed by the demonstration of its localization in the nucleolus and telomeres [13] and low histone acetylation level in the genetic loci highlighted the importance of this group of proteins in regulating chromatin structure in specific loci. These findings were followed by demonstration of the importance of the protein in regulating yeast lifespan [14] and identification of Sir2 as an NAD-dependent histone deacetylase [15]. This revealed the connection between a molecule involved in gene

silencing and cellular metabolism and led to establishing sirtuins as important epigenetic regulators. This was followed by identification of *Sir2* orthologues in mammalian systems; these were demonstrated to be NAD^+-dependent protein deacetylating enzymes that are highly conserved across bacteria to humans.

On the basis of sequence similarity, mammalian sirtuins are classified into four classes, class I–IV. Mammalian SIRT1–3 belong to class I, SIRT4 to class II, SIRT5 to class III and SIRT6 and 7 to class IV (**Table 1**) [9, 16]. All these enzymes share a conserved catalytic core structure, but they differ in their enzymic activity. While class I sirtuins (SIRT1–3) show robust deacetylase activity in vitro, SIRT4–7 show weak deacetylase activity; SIRT4 shows mono ADP–ribosyl transferase activity and SIRT2, 3 and 6 exhibit both deacetylase and ribosyl transferase activity. SIRT5 removes succinyl, malonyl and glutaryl groups from acylated protein–lysine residues. SIRT6 is more efficient in removing long chain fatty acyl groups such as myristoyl and palmitoyl groups. The enzymic reaction proceeds through the formation of a ternary complex involving acetyl protein substrate and NAD at the active site, which decomposes to form deacylated protein, nicotinamide and 2-*O*-acetyl-ADP ribose. They also differ in tissue distribution and subcellular localization (**Table 1**) [6, 9]. SIRT1 is expressed in metabolic tissues such as liver, muscle, adipose tissue and other organs such as heart, brain, pancreas; it is localized mainly in the nucleus, but shuttles from nucleus to cytosol. SIRT2, primarily a cytosolic protein, is highly expressed in heart, brain and skeletal muscle. SIRT3–5 are expressed in mitochondria. SIRT3 and 5 are mainly expressed in kidney, brain, liver and heart. In contrast to white adipose tissue, brown adipose tissue (BAT) expresses SIRT3. SIRT4 is expressed in heart, liver, pancreas and vascular smooth muscle. SIRT6 and 7 are localized predominantly in the nucleus. While SIRT6 is expressed in brain, liver and muscle, SIRT7 is mainly found in liver and spleen.

2.1.2. Biological effects of sirtuins

Mammalian sirtuins influence various cellular processes such as chromatin silencing, cell cycle regulation, differentiation and survival, mitochondrial biogenesis, metabolism, inflammation and stress response. While most studies in model organisms indicate a role for sirtuins in mediating the increased longevity affected by calorie restriction [17], a similar role for mammalian sirtuins is debated [18]. Sirtuins deacetylate transcription factors and regulate their activities either by influencing their cytoplasmic-nuclear distribution, their binding to DNA or changing their interaction with regulatory proteins.

SIRT1 is by far the best characterized among all mammalian sirtuins. It has been linked to hypothalamic control of energy balance [19], has a role in adipogenesis and fat mobilization, as well as regulation of carbohydrate and lipid metabolism [6, 7]. SIRT1 promotes vasodilation and regenerative function in endothelial and smooth muscle cells of vascular wall by targeting eNOS for deacetylation [20]. In cardiomyocytes, SIRT1, 3 and 7 play a critical role in promoting cardiomyocyte resistance to stress and toxicity [21].

Important targets of SIRT1 include p53, forkhead box type O transcription factors (FOXO), PPARγ co-activator-1α (PGC1α), NFkB, androgen receptor and their co-regulatory molecules (**Table 1**). Apart from its effect on PGC1α, a master regulator of mitochondrial biogenesis,

	Sirt 1	Sirt 2	Sirt 3	Sirt 4	Sirt 5	Sirt 6	Sirt 7
Class	I	I	I	II	III	IV	IV
Subcellular location	Nucleus, cytoplasm	Cytoplasm, nucleus	Mitochondria	Mitochondria	Mitochondria	Nucleus	Nucleolus
Tissue	Liver, heart, brain, pancreas, muscle, adipose tissue	Heart, brain, skeletal muscle	Brown adipose tissue, kidney brain, heart, liver	Vascular smooth muscle, skeletal muscle, heart, liver, pancreas	Brain, heart, muscle, liver, kidney	Brain, liver, muscle	Liver, spleen
Activity	Deacetylation	Deacetylation	Deacetylation	ADP-ribosylation	Deacetylation, demalonylation desuccinylation	Deacetylation, ADP-ribosylation	Deacetylation
Target	PGC1-α, PPARγ, PPARα, FOXO1, FOXO3, p53, notch, NF-kB, HIF-1α, LXR, FXR, SREBP1c	Tubulin, PEPCK, FOXO-1,PAR-3	LCAD, HMGCS-2, SOD-2, GDH, IDH2	GDH, Malonyl CoA decarboxylase	CPS1	H3KK9, H3K56	SIRT 1
Biological effects (WAT)							
Adipogenesis	Inhibition	Inhibition					Stimulates
Lipogenesis	Inhibition (decreases AC1)			Stimulates malonyl decarboxylase			
Lipolysis	Stimulates (FOXO1, ATGL)	Stimulates					
β-oxidation							
Oxphos							
Glucose metabolism	Improves insulin sensitivity				Decreases insulin sensitivity		
Inflammation	Decreases				Decreases		Increases
Adipokines	Increases adiponectin Decreases leptin		Increases adiponectin			Increases adiponectin	Decreases leptin

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	Sirt 1	Sirt 2	Sirt 3	Sirt 4	Sirt 5	Sirt 6	Sirt 7
Biological effects (BAT)							
Brown adipogenesis			Stimulates (expressed more in BAT than WAT)		Stimulates (expressed more in BAT than WAT)		Inhibits (expressed more in BAT than WAT)
Browing	Stimulates						
Whitening	Inhibits						
β-oxidation	Stimulates		Stimulates				
Oxphos	Stimulates mitochondrial biogenesis		Stimulates				
Thermogenesis	Stimulates		Stimulates				Inhibits
Glucose metabolism	Improves glucose tolerance			Increases on cold exposure		Increases glucose uptake	Decreases glucose tolerance
Effects on sirtuins							
Obesity	Decreased		Decreased	Increased	Decreased?	Decreased	Increased/ decreased
CR	Increased		Increased	Decreased (?)		Increased	Increased
Gastric banding surgery	Increased			Increased			
References	[6, 7, 11, 31, 52, 53, 77, 78, 100, 102]	[6, 7, 11, 26, 31]	[6, 7, 11, 27, 31]	[6, 7, 11, 31, 65, 66]	[6, 7, 11, 31]	[6, 7, 11, 31]	[6, 7, 11, 31, 108]

Table 1. Sirtuins – targets, enzymic activity and biological effects.

SIRT1 acts on several transcription factors like estrogen like receptors, the nuclear respiratory factors 1 and 2 to induce mitochondrial gene expression [22].

SIRT1 appears to directly block lipid anabolism by interfering with PPARγ and LXR signaling. The repressive effect of SIRT1 on PPARγ activity requires the formation of a co-represssor complex that involves NCoR1 [23]. SIRT1 also has a role in reverse cholesterol transport; it stimulates cholesterol efflux from macrophages and the hepatic conversion of cholesterol to bile acids through LXR [24] . It is also present in the cytosol of many cell types and regulates major cytoplasmic enzymes such as acetyl CoA synthase and eNOS by deacetylation [20].

Compared to SIRT1, not much is known about the physiological effect of other mammalian sirtuins. SIRT2 shows similarity to SIRT1 in several of its biological effects. It appears to increase hepatic glucose which is beneficial in starving conditions where SIRT2 activity increases. While it suppresses glycolysis by deacetylating and destabilizing glucokinase, it activates gluconeogenesis by enhancing the action of a key gluconeogenic enzyme PEPCK by its deacetylation [25]. It also appears to have a role in the control of microtubule stability and cell cycle oscillations by deacetylating α-tubulin [26].

SIRT3 is a major regulator of mitochondrial function; it deacetylates several mitochondrial proteins which are critical in mitochondrial oxidative metabolism. Calorie restriction increases activity of SIRT3 which alters the mitochondrial acetylome [27, 28]. SIRT4 is a mitochondrial matrix protein with remarkable ADP-ribosyltransferase activity [29]. It is reported to regulate insulin secretion [30], the activity of glutamate dehydrogenase, and serves as a metabolic regulator by inhibiting the activity of several metabolic enzymes such as pyruvate dehydrogenase opposing the effect of SIRT3 [31]. Although recent studies have identified several SIRT5 target proteins, not much is known about its biological function. SIRT5 appears to play a role in energy homeostasis and free radical metabolism [32]. SIRT6 deficient mice aged prematurely and its overexpression increased life span of male mice apparently by altering IGF signaling [31].

2.1.3. Regulation of sirtuins

Sirtuins are regulated in response to nutritional and metabolic challenges, oxidative stress, and inflammation in a cell and tissue specific manner. They are subject to transcriptional control, post-transcriptional regulation by miRNA and post-translational modulation [31]. These regulatory events can either alter the levels of each sirtuin or their enzyme activity or both. Their activity can be modulated either directly by post-translational modifications(PTMs) such as phosphorylation and acetylation, protein interactions and by compounds that activate them, or indirectly by modulating NAD^+ levels. The substrates themselves appear to regulate sirtuin expression indicating the possible formation of feedback regulatory loops.

Some of the key factors involved in metabolic homeostasis that cause upregulation of SIRT1 include CREB, FOXO1, FOX3a, C/EBPα, PPARα and PPARβ/δ while the negative regulators include ChREBP, C/EBP. NFkB, EGR1, APE1 positively regulate transcription of SIRT1 during stress conditions. SIRT1 in turn can modulate the activity of several of these transcription factors. The enzymatic activity of SIRT1 is enhanced by post-translational modification by phosphorylation [33] and SUMOylation (Lys734) [34]. The activity of SIRT1 can also be controlled

through interaction with different protein complexes such as DBC1 (nuclear protein deleted in breast cancer-1), AROS (active regulator of SIRT1) and NCoR1 [35, 36]. SIRT1 can also be activated indirectly by either increasing NAD^+ synthesis from NAD^+ precursors like nicotinic acid, NAM, nicotinamide riboside or decreasing NAD^+ consumption by two alternate enzyme families, PARP and cADP ribose synthase [37]. SIRT1 can enhance its own activity by auto-deacetylation. This is inhibited by SIRT7 suggesting coordinated action of different sirtuins [38]. SIRT1 is inhibited by NADH which competes with NAD^+ [39] and by nicotinamide [40]. *In vitro* studies using malignant cell lines, have reported the role of different miRNAs in the post-transcriptional regulation of SIRT1 [31]. Mir34a is reported to regulate SIRT1 at different levels; it inhibits translation of SIRT1 mRNA; it indirectly suppresses transcription and enzyme activity of SIRT1 by modulating the levels of NAD through regulation of the biosynthetic enzyme nicotinamide phosphoribosyl transferase [41].

Unlike SIRT1, information on the regulation of other sirtuins is limited. Cyclin(E-Cdk2 and A-Cdk2) mediated phosphorylation at S331 inhibits SIRT2 activity whereas Erk1/2 enhanced its activity [31]. Cyclic AMP–PKA pathway, which is activated in response to various stimuli, causes upregulation of SIRT3 expression through PGC1α. cAMP may also activate SIRT3 by direct binding [31]. Activated AMPK also positively regulates SIRT3 by increasing NAD. Similar to SIRT3, expression of SIRT5 is also upregulated by PGC1α, while AMPK appears to negatively regulate its expression [31]. Several transcription factors and miRNAs appear to regulate expression of SIRT6. While Nuclear Respiratory factor 1 (NRF-1) and co-activators induce SIRT6 expression during nutrient deprivation, cAMP signaling reduces SIRT6 expression. A relation between SIRT6 and mir122 has also been suggested [31].

2.2. Sirtuins regulate WAT development and metabolism

Adipose tissue is classified into white adipose tissue (WAT) which serves as the principal energy storage organ and brown adipose tissue (BAT) whose principal function is maintaining temperature by non-shivering thermogenesis. A third category includes beige or brite (brown in white) adipocytes within WAT which can potentially differentiate into cells of brown like phenotype.

2.2.1. Sirtuins and WAT development

The cellular and molecular mechanisms that govern the adipocyte life cycle have been extensively studied [42–45]. Both white and brown adipocytes develop by a highly regulated process of differentiation of mesenchymal stem cells (MSC). During the early phase of adipogenesis the pluripotent MSCs are committed to unipotent pre-adipocytes which in the latter phase undergo terminal differentiation acquiring the characteristic phenotype and functions of mature adipocytes. The complex process of adipocyte differentiation is coordinated by myriad factors. Isoforms of bone morphogenetic protein, BMP2 and BMP4 are the key positive regulators of commitment to white pre-adipocytes [46, 47]. This early phase of differentiation is also subject to negative regulation by several transcription factors including members of GATA and Forkhead family, Wnt and Notch signaling, Kruppel-like factors 2 and 7 (KLF2, KLF7) and CHOP proteins [48].

The differentiation of white pre-adipocytes to mature adipocytes is mediated by multiple transcription factors. The key transcription factors involved are CCAAT enhancer binding protein (C/EBP), PPARγ and sterol-regulatory element binding protein 1 (SREBP1) [43]. C-EBP, which exists in six different isoforms, is activated and translocated to the nucleus in response to cAMP mediated signaling [43]. Hormones induce transient expression of C/EBPβ and C/EBPδ which upregulate expression of PPARγ and C/EBPα [43] PPARγ, (particularly the isoform PPARγ1) that belongs to the nuclear receptor superfamily, heterodimerises with retinoid receptor α (RXRα), another nuclear receptor, and binds to DNA to promote expression of adipocyte specific genes such as leptin, adiponectin, FABP4, and perilipin. Activation of C/EBPα leads to transcriptional activation of several genes encoding proteins such as GLUT4, SCD1, FABP4 which are critical in establishing adipocyte phenotype [43].

All seven sirtuin genes are expressed in human and rodent WAT. A difference in their level of expression, particularly a reduction in SIRT1, has been observed, in experimental obese models as well as obese human subjects [11]. Studies in experimental model systems suggest that sirtuins, particularly SIRT1, are negative modulators of WAT adipogenesis. Overexpression of SIRT1 decreased accumulation of fat while its knockdown increased fat accumulation in 3T3L1 cells undergoing differentiation [23]. Resveratrol, an activator of SIRT1 reduces osteoblastic differentiation of MSC to adipocytes [49]. SIRT1 is upregulated in WAT in calorie-restricted mice model in which there was significant reduction in fat mass [50, 51]. Further, transgenic mice overexpressing SIRT1showed lower body weight and reduction of fat mass [52], while ablation of SIRT1 in WAT resulted in gain in body weight, increase in fat mass and an increase in the size of individual adipocytes [53]. Studies using bone marrow-derived MSC with SIRT1 deletion showed impaired self- renewal and differentiation to osteoblasts without significantly affecting their differentiation to adipocytes [54]. In the light of these observations it has been suggested that although SIRT1 inhibits adipocyte differentiation, its expression is critical for maintenance of MSC pool [11].

PPARγ is an important substrate for SIRT1 [55]. SIRT1 dependent deacetylation of lysine residues (268 and 293 K) on PPARγ is critical in the regulation of its transcriptional activity by its co-repressors NCoR and SMRT. Sirt1 can thus inhibit white adipogenesis by suppressing the transcriptional activity of PPARγ by promoting the binding of its co-repressors NCoR and SMRT [23]. Further, C/EBPα, a PPARγ dependent factor, regulates the expression of SIRT1 during adipogenesis [56]. miRNAs can also regulate effects of SIRT1 on adipogenesis. For example, mir 146b can promote adipogenesis by suppressing SIRT1-FOXO1 cascade [57]. SIRT2, the predominant sirtuin in adipose tissue, has also been shown to inhibit adipocyte differentiation by deacetylating FOXO1 and enhancing its repressive interaction with PPARγ [58, 59]. Unlike SIRT1 and SIRT2, SIRT7 knockdown in human pre-adipocytes reduced lipid content and the number of $FABP4^+$ differentiated adipocytes indicating a contrasting effect of SIRT7. Further, SIRT7 knockout mice had significantly reduced WAT and increased SIRT1 activity. This suggests that SIRT7 influences adipogenesis in mice by inhibiting autocatalytic activation of SIRT1, further highlighting the importance of cross talk among sirtuins in the control of adipose tissue maintenance [38].

2.2.2. Sirtuins and WAT metabolism

During times of positive energy balance, storage of lipids as triglycerides in lipid droplets in adipocytes leads to expansion of adipose tissue. It leads to changes in the levels of adipokines such as leptin and adiponectin that affect metabolic functions of other organs particularly liver, muscle and brain, which in turn can affect the adipose tissue function [43, 45]. The stored lipid is mobilized in response to hormones and systemic energy needs during periods of negative energy balance. Metabolism of adipose tissue is thus linked with that of other tissues. SIRT1 appears to serve as an important metabolic switch through which adipose tissue and other metabolic organs respond to energy needs [8].

When energy stores are high, excess nutrients particularly glucose and amino acids are used to synthesize fatty acids de novo primarily in liver and are exported to WAT where they are stored as TG [6] SIRT1 deacetylates sterol-responsive element binding protein 1c (SREBP1c) causing reduction of its transcriptional ability and suppression of fatty acid synthesis [6]. Further, by increasing PGC1α-mediated mitochondrial biogenesis, SIRT1 facilitates fatty acid oxidation. SIRT1 promotes gluconeogenesis in the liver through deacetylation and activation of PGC1α and FOXO1, and increasing expression of gluconeogenic enzymes [22] and inhibits glycolysis [6] This increases hepatic production of glucose during fasting. Apart from SIRT1, SIRT6 also has been shown to repress glycolysis in liver [31]. Further, SIRT1, by deacetylating STAT3, reduces its repressive effect on gluconeogenesis [60].The hepatic effects of sirtuins can thus affect nutrient flux into the adipose tissue.

Sirtuins are not only critical in WAT adipogenesis, they also play a key role in maintaining the functions of differentiated adipocytes by regulating the expression of several PPARγ-responsive genes involved in metabolism. Activation of SIRT1 reduces expression of acetyl coA carboxylase and other lipogenic genes involved in the de novo synthesis of FAs in pre-adipocytes. Multiple studies support the role of PPARγ in regulating adipose tissue metabolism [61]. Over-expression of a dominant negative form of PPARγ downregulates the expression of key genes involved in lipid metabolism, insulin signaling and decreases lipid content in 3T3L1 differentiated adipocytes [62]. Further, selective ablation of PPARγ in mature white and brown adipocytes results in adipocyte death without any effect on pre-adipocyte differentiation indicating the requirement of PPARγ for maintenance of differentiated functions of adipocytes [63]. Insulin dependent glucose uptake in the adipocyte takes place through the GLUT4 transporter. Expression of GLUT4 gene is regulated by PPARγ. SIRT1 appears to regulate glucose–induced secretion of insulin by transcriptional repression of UCP2 which uncouples mitochondrial ATP production [64]. SIRT1 by deacetylating SREBP1, destabilizes and reduces its occupancy on the lipogenic gene promoters suppressing fatty acid synthesis [6]. Although SIRT1 inhibits lipogenesis in WAT, SIRT4 appears to have an opposite effect. Deletion of mitochondrial SIRT4 decreased lipogenesis apparently by regulating malonyl CoA decarboxylase, thereby altering the level of malonyl CoA that represses fatty acid oxidation [65, 66]. While an increase in SIRT4 in fed state results in deacetylation and inactivation of malonyl CoA decarboxylase resulting in enhanced FA synthesis, during fasting, reduction of SIRT4 expression

leads to activation of malonyl CoA decarboxylase resulting in enhanced lipid oxidation. It therefore appears that SIRT4 exhibits a dual effect regulating anabolism and catabolism of fatty acids [65]. SIRT6 also appears to regulate lipogenesis. In SIRT6 overexpressing mice, expression of diacylglycerol acyl transferase a key enzyme involved in TG synthesis was down regulated along with certain PPARγ responsive genes involved in lipogenesis [31, 67].

2.2.3. Mobilization of depot fat

During periods of negative energy balance, the triglycerides stored in the lipid droplets, are hydrolysed to FFA which are released into circulation. In the basal state or during TG synthesis, perilipin, the structural protein on lipid droplet, is bound to protein CGI-58 (comparative gene identification-58) which is a co-activator of the adipose triglyceride lipase (ATGL). In response to cAMP dependent PKA-mediated phosphorylation of perilipin, CGI-58 is released leading to activation of ATGL [68]. Activated ATGL moves to the lipid droplet membrane surface to hydrolyse TG to diacyl glycerol. Further activation of hormone sensitive lipase (HSL) by PKA mediated phosphorylation causes binding of HSL to perilipin and continues lipolysis forming monoacyl glycerol which is acted upon by another specific monoacyl glycerol lipase (MGL) forming glycerol and FFA which are released to plasma for systemic utilization.

Unlike its suppressive effect on adipogenesis in WAT, SIRT1 appears to promote mobilization and utilization of depot fat. Overexpression of SIRT1 in differentiated 3T3L1 cells resulted in decreased triglyceride levels and increased release of FFAs [23], while knockdown of SIRT1 decreased basal and stimulated lipolysis in adipocytes in culture. In *in vivo* studies using mice receiving high fat diet, activators of SIRT1 such as resveratrol reduced fat mass [69]. Further, over expression of SIRT1 inhibited diet induced accumulation of fat [6, 31, 52]. SIRT1 regulates the expression of ATGL gene and thereby lipolysis in adipocytes through modulation of the acetylation and transcriptional activity of FOXO1 [70]. SIRT2 also appears to show similar effect on fat mobilization [58, 59].Although sirtuins influence mitochondrial oxidative metabolism in other metabolic tissues such as liver, their role in WAT mitochondrial metabolism is poorly understood.

2.3. Role of sirtuins in BAT development and browning of WAT

Unlike white adipogenesis, BAT biogenesis involves BMP7-stimulated commitment of Myf 5^+ cells to brown pre-adipocytes that mature into mitochondria–rich brown adipocytes.BMP7 stimulation of progenitor cells leads to down regulation of early adipogenic inhibitors such as Pref1, Nectin, Wnt signaling molecules [43]. This is followed by upregulation of transcription factors such as PPARγ and C/EBPα which cause upregulation of expression of PRDM16, a key factor involved in adipocyte–myocyte switch through activation of expression of BAT specific genes (PGC1α, UCP-1, ZIC1) and downregulation of myogenic genes such as Myf5 and MyoD or myogenin [43]. This leads to increased biogenesis of mitochondria. PRDM16 is a 140 kDa protein that binds and activates the transcriptional function of PPARγ and PGC1α. PGC1α co-activates PPARγ-RXRα heterodimer stimulating the expression of BAT-specific genes UCP1and UCP3.

The ability of human white adipocytes to acquire brown fat-like phenotype, termed browning, in response to β-adrenergic stimulation, cold exposure [43] and by several molecules such as muscle derived irisin [71, 72] liver derived FGF21 [73] and small molecules such as β-aminoisobutyric acid [74] has been observed. These type of cells called beige or brite adiopcytes express genes involved in thermogenesis such as UCP1, deiodinase type II and PGC1α in response to stimulation of β3–adrenergic receptors [43].

Sirtuins also appear to play a role in the differentiation and function of BAT. Like WAT, brown adipose tissue also has been shown to express all the members of the mammalian SIRT family [11]. While the relative level of expression of SIRT3 and 5 are higher, that of SIRT1 and 7 are lower in BAT than those in WAT. Calorie restriction (CR) and cold exposure upregulated the expression of SIRT3 present in the mitochondria in BAT [75]; SIRT1 and SIRT2 also showed an upregulation under such conditions. Conversely, SIRT3 is down regulated in BAT in high fat diet induced obese mice. SIRT1 also appears to have a role in differentiation of pre-adipocyte to brown adipocytes. It appears that SIRT1 influences BAT differentiation through repression of the MyoD-mediated myogenic gene expression signature and stimulation of PGC-1α mediated mitochondrial gene expression [76]. SIRT1 appeared to improve glucose homeostasis in SIRT1 transgenic mice and brown adipocytes derived from them due to an enhanced response of brown adipocytes to β3-adrenergic stimuli rather than differences in differentiation status [77]. Of the different sirtuins, SIRT3 appeared to be critical in the differentiation of brown adipocytes. SIRT3 has been shown to activate PGC1α mediated thermogenic response in differentiating brown adipocytes.

Brown remodeling of white fat in response to cold exposure is shown to be regulated by SIRT1-dependent deacetylation of PPARγ. SIRT1-dependent deacetylation of Lys 268 and Lys 293 of PPARγ is required to recruit the co-activator PRDM16 to PPARγ, leading to upregulation of BAT-specific genes and repression of WAT genes [78].In response to different environmental stimuli, SIRT1 can differentially modulate PPARγ in WAT. SIRT1 inhibits PPARγ through local modulation of acetylation status of histones and recruitment of co-repressor NCoR in response to caloric restriction; but on cold exposure, it directly enhances PPARγ signaling through deacetylation of PPARγ itself [79]. SIRT1 deficiency in mice results in accumulation of lipid droplets and reduction of mitochondrial content in BAT indicating a role for SIRT1 in the white remodeling of BAT which appears to occur in obese conditions [80].

2.4. Sirtuins and obesity

Grossly elevated fat stores in adipose tissue with hypertrophic or hyperplastic adipocytes and concomitant development of blood vessels result in obesity. The relevance of sirtuins in adipose tissue development and metabolism and their effects on metabolism of glucose and lipids primarily in the liver, and insulin function suggest a possible link between sirtuins and obesity.

The level of expression and activity of SIRT1 decrease in adipose tissue in different obesity models. Expression of SIRT1 in adipose tissue of db/db leptin resistant obese mice and in mice fed on HFD was significantly low [53, 81]. Overexpression of SIRT1 in HFD-induced obese animals caused less inflammation and better glucose tolerance. SIRT1 expression in obese pigs

is reported to be lesser than that in lean pigs [82]. Apart from decrease in SIRT1 levels, its function is also affected by changes in its post translational modification in obesity. One of the important post-translational modifications of SIRT1 which has been shown to be affected in obesity leading to inhibition of its nuclear localization is casein kinase mediated phosphorylation of ser-164 which is enhanced in obese and not in lean animals [83]. Unlike SIRT1 which is decreased in WAT in obesity, there is no consensus on the changes in other sirtuins in obese WAT; while some reports show decrease in SIRT2–6, other reports do not show any significant differences between obese and respective controls. But in obese BAT, SIRT1 and SIRT3 are down regulated and SIRT7 is upregulated. It has been shown that mir34a, which regulates the expression and activity of Sirt1, is elevated in obesity [41]. A possible association of sirtuins with obesity and obesity-associated pathological conditions in humans has also been indicated mostly from observational studies [84–88]. There is significant reduction in sirtuins in adipose tissue and other metabolic tissues in obese subjects and that weight loss or long term fasting can result in increase in their expression.

2.4.1. Sirtuins, insulin response and energy homeostasis

Insulin resistance is a hallmark of obesity and a major factor contributing to obesity associated pathological conditions. *In vitro* and *in vivo* studies suggest that SIRT1 regulates insulin response. In insulin resistant cells where SIRT1 is down regulated, induction of SIRT1 expression increased insulin sensitivity [89]. SIRT1 regulated insulin-dependent glucose uptake in adipocytes. Increase in SIRT1 activity improved insulin sensitivity [90]. Adipose tissue-specific SIRT1 knockout mice were reported to be more prone to developing insulin resistance. In experimentally induced diabetic animals, overexpression of SIRT1 increased insulin sensitivity. Mechanistically, SIRT1 effect appears to involve transcriptional repression of protein tyrosine phosphatase 1B gene which is critical in insulin signaling [91] Along with SIRT1in WAT, SIRT3 and SIRT5 contribute to systemic glucose homeostasis. As indicated before, SIRT1 also regulates insulin secretion by β-cells of pancreas by repressing UCP-2 [64]. Inhibition of SIRT1 expression reduced insulin secretion in β-cell lines; conversely overexpression of SIRT1 increased it. *In vivo*, transgenic mice over expressing SIRT1 in pancreatic β-cells showed increase in glucose-stimulated insulin secretion [92]. Further, SIRT1 deficiency impaired insulin secretion apparently by disrupting glucose sensing and impairing response to fluctuations in glucose levels [93].

In addition to its effect on peripheral tissue metabolism, SIRT1 in hypothalamus appears to act as a key regulator of central control of energy homeostasis. Evidence in support of this include (a) increase in the expression and activity of SIRT1 in hypothalamus in both calorie restriction and fasting [19, 94] (b) inhibition of hypothalamic SIRT1 expression, specifically in anorexigenic POMC neurons, resulted in loss of response to leptin and reduced energy expenditure indicating requirement of SIRT1 in POMC neurons for homeostatic defense against diet- induced obesity [95] (c) deletion of SIRT1 expression specifically in orexigenic Agouti-related peptide (AgRP)-expressing neurons, which promotes feeding in response to fasting, decreased AgRP neuronal activity resulting in decreased food intake and body weight [96] (d) Central inhibition of SIRT1 in rodents on a high fat diet caused decreased body weight and increased energy expenditure. This is mediated through increased acetylated-FoxO1-mediated increased production of POMC and its active product αMSH which in turn augmented TRH and T3 levels suggesting a hypothalamic–pituitary-thyroid axis which stimulates energy expenditure [97].

2.4.2. Sirtuins and inflammation in adipose tissue

Inflammation of adipose tissue in obesity is a major contributor to insulin resistance and pathogenesis of the metabolic syndrome [98]. SIRT1 could act as a transcriptional regulator of inflammation in multiple tissues, particularly adipose tissue as well as macrophages and endothelial cells [90, 99]. The levels of SIRT1 is inversely related to inflammation in adipose tissue. SIRT1 expression in human subcutaneous adipose tissue was less in cases where macrophage infiltration was high [100–102]. The decrease in SIRT1 in obese conditions in adipose tissue is suggested to be due to its proteosomal degradation. Activation of C-jun N terminal kinase (JNK1), which is a key component in inflammation associated signaling pathway, leads to phosphorylation of SIRT1, followed by its degradation in proteasomes [53, 103]. The molecular basis of the beneficial effect of SIRT1 on inflammation is related to suppression of NFkB activation [104]. SIRT1 inhibits transcriptional activity of NFkB directly by deacetylating the RelA/p65 subunit of NFkB at Lys 310 [105]. Moreover mir34a dependent decrease in SIRT1 activity can increase NFkB activity [41]. This suggests that SIRT1 and inflammatory signals interact at various levels and that SIRT1 is an important molecular link between nutrients, inflammation and metabolic dysfunction of the tissue. Though not much data on the role of other sirtuins in inflammation in human subjects is available, SIRT5 expression levels also correlate inversely with markers of inflammation [106] Deletion of SIRT7 in mice reduced WAT inflammatory gene expression in HFD induced obesity, suggesting opposing functions for SIRT1 and 7 [107].

2.4.3. Sirtuin activators for therapy

Since sirtuins play an important role in regulation of adipogenesis, and adipose tissue metabolism, pharmacological activation of sirtuins could be a useful approach for the treatment of obesity and related metabolic disorders. Sirt1 is an allosteric enzyme which is regulated by ligand binding. Resveratrol, which is a naturally occurring polyphenol with anti-oxidant property, increased the enzyme activity of SIRT1 by binding to its allosteric site [108]. High throughput screening has identified several small molecular activators of SIRT1 [109]. The most potent of these is SRT1720 which, protects against diet induced obesity [6]. The administration of SRT1720 reduced expression of lipogenic enzymes and reduced hepatic lipid accumulation, it also enhanced oxidative metabolism in skeletal muscle, liver and BAT in mice, protecting from HFD induced obesity and insulin resistance [110]. However, increasing sirtuin activity could result in indiscriminate deacetylation of histones and several other key proteins in different tissues. Sirtuin activating therapies would therefore have to be target specific.

3. Conclusions

Epigenetic modifications have emerged as fundamental modulators of metabolic functions, and sirtuins, a group of class III histone deacetylases, play a key role in this context. In this chapter the regulatory effects of sirtuins on adipose tissue metabolism in both WAT and BAT, and implication of alterations in their expression and activity in obesity, inflammation, and insulin resistance have been highlighted.

In spite of considerable advances in molecular biology of mammalian sirtuins, many questions remain unanswered. Among the different sirtuins, the role of SIRT1 in development and metabolism of white adipose tissue is reasonably well known. Although all the other sirtuins are expressed in WAT, their role in WAT function is not clear. Similarly, the role of SIRT1 and 3 in brown remodeling of white fat has been elucidated, but the regulatory effects of the other sirtuins are still unknown.

Different sirtuins control similar cellular processes in adipose tissue. Unraveling the potential crosstalk and coordination between them will require further study. The significance of this in the possible gene regulatory network and coordinated action among sirtuins in metabolic regulation is evident from the antagonistic interaction between SIRT1 and 7 in adipose tissue metabolism.

Apart from functional differences between WAT and BAT, variations in the status of different depots of WAT are also related to a risk for obesity-associated diseases. It is not clear whether there is any depot dependent variation in sirtuin action.

As the expansion of a vascular tissue like adipose tissue is associated with neovascularization, adipogenesis and angiogenesis are interrelated. Understanding the role of sirtuins in adipose tissue angiogenesis is of paramount importance especially in brown tissue, where both the mitochondrial activity and oxygen demand are high.

Sirtuins appear to be an attractive target for the treatment of obesity and related metabolic disorders. Increasing sirt activity in adipose tissue by identifying natural compounds, or engineering small molecular activators is an area which needs intensive research; increasing intracellular levels of NAD^+, a substrate for sirtuins, is an alternate approach.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Chapter 4

Browning of Adipose Tissue and Sirtuin1 Involvement

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Additional information is available at the end of the chapter

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Abstract

Obesity is an important risk factor for many diseases, including cardiovascular diseases, metabolic syndrome and cancers. Excessive dietary intake of caloric food results in its accumulation in white adipose tissue (WAT), whereas energy expenditure by fat utilization and oxidation predominately occurs in brown adipose tissue (BAT). Reducing obesity has become an important prevention strategy of research interest, focusing in the recent years, mainly on browning of WAT, the process during which the enhance of the mitochondria biogenesis occurs and then white adipocytes are converted to metabolically active beige adipocytes. Sirtuin1 (SIRT1), the most known isoform of sirtuin deacetylases, is implied in the browning of WAT process. In fact, it is a sensitive sensor of cell energy metabolism and, together with other sirtuin isoforms, contributes to this differentiation process. This chapter provides an overview about SIRT1 involvement in browning of WAT as a target molecule that can thereby contrast obesity.

Keywords: adipose tissue, browning, obesity, resveratrol, sirtuin1

1. Introduction

Lipids are stored in the body by two types of adipose tissue such as white adipose tissue (WAT) and brown adipose tissue (BAT) [1–3]; the main parenchymal cells of the adipose tissue are adipocytes, and so far two fat cell types, white and brown cells, have been reported, respectively [4–6]. The white and brown adipocytes arise from separate progenitor cell lines, present distinct structure, morphology, localization and functions [1, 5, 7] and these differences contribute to the maintenance and modulation of energy and of metabolic health [4, 8, 9].

Despite these differences, both types of adipocytes share the activity of accumulation and release of fatty acids and both express the rather specific adrenergic receptor β3 [10, 11].

The adipose tissue is highly dynamic in the sense that changes in mass of an order of magnitude can occur, this happen during physiological (like pregnancy/lactation or aging) and pathological states (like metabolic syndrome, obesity, etc.) [12, 13]. In physiological condition, functional WAT and BAT adipose tissues control and modulate the energy balance with important effects on metabolic health and longevity [6, 14]. Aging is typically associated with a body redistribution of adipose tissue (increased central, visceral and ectopic adiposity) [4, 6, 15, 16] and chronic low-grade inflammation [17, 18]. Aging is also related to an increase of lipotoxicity due to the reduced capacity of adipose depots to store free fatty acids [16, 19]. These conditions contribute to increased risk for metabolic perturbations such as insulin resistance, impaired glucose tolerance and diabetes [18, 20]. Of importance, key processes of adipose tissue physiology affect molecular pathways that regulate lifespan [21], such as sirtuins (SIRTs) levels decline with age in several tissues, including adipose tissue, and this reduction induces adipocyte dysfunctions leading to obesity [6, 22].

To date, in both advanced and developing countries, obesity has become a major health problem [7, 12, 23], mainly because it carries an increased risk of death for the associated disorders [24, 25]. In fact, excess WAT throughout the body is associated with an increased risk of cardiovascular diseases, breast, colon, oesophageal, gall bladder and pancreatic cancers, sleep apnea and physical disabilities, such as knee arthritis [26–28].

In the following sections, we present firstly the main differences between WAT and BAT and the WAT browning process and then we discuss the potential SIRT1 involvement in this process as a target molecule that can thereby contrast obesity, introducing also the effects of resveratrol as a SIRT1 exogenous inducer.

2. White adipose tissue

WAT is now known to be highly dynamic, synthesizing and secreting multiple lipids, proteins and autocrine, endocrine, paracrine and neuroendocrine factors which are involved in the regulation of a wide range of physiological and metabolic processes [4, 29]. In particular, white adipocytes, characterized by a large unilocular lipid droplet, have a low density of mitochondria and variable size (25–200 μm) [6, 7, 12, 29, 30] and they might secrete adipokines that are pro- and anti-inflammatory cytokines fundamental in the regulation of metabolic functions and also in the communication from adipose tissue to other tissues [7, 12, 31]. Other lipid molecules are also secreted by WAT, including prostanoids, cholesterol and retinol, which are stored in order to be subsequently released [4, 32].

The development of subcutaneous WAT begins in uterus, but primarily occurs after birth, when specialized fat storage cells are needed to provide fuel during fasting periods. Hyperplastic and hypertrophic white adipocyte processes occur during WAT development, throughout the organism's lifespan and in conditions of positive energy balance [33–35]. If the caloric excess is not reconciled by increased energy utilization, cellular hypertrophy and hyperplasia occur and then lead to adipocytes dysfunctions and so obesity [36, 37].

3. Brown adipose tissue

Since the 1970s, BAT has been increasingly recognized as the main site of nonshivering thermogenesis in mammals [35, 38] and it is probably the outcome of a single evolutionary development, in fact, unlike WAT, BAT is only found in mammals [35]. BAT adipocytes are smaller (15–60 μm) than white adipocytes [39] and present a characteristic brown color due to its high content of mitochondria and a lobulated surface that is innervated and very well vascularized [6, 8, 30]. BAT has the physiological role of metabolizing fatty acids in order to produce heat [4, 26], that is why it is often referred to as "good" fat, since it helps burn, not store, calories. This specific role of BAT is supported by the high content in its mitochondria of uncoupling protein1 (UCP1), uniquely expressed in these cells [4, 8, 26, 40]. The activation of UCP1 and transcriptional induction of the genes encoding UCP1 induce uptake of lipids and glucose from the circulation to sustain oxidation and thermogenesis [35, 41].

Functional BAT is more common in women than men and its mass and activity are reduced in overweight, obese and aging people [4, 41, 42]. Thus recruitment and activation of BAT seem to be a good tool to counteract obesity and its related diseases.

4. Browning of white adipose tissue

Currently, the terms browning, britening and beiging are used as synonyms to describe the differentiation of white adipocytes from brown adipocytes, so defined beige fat cells [30, 39, 43–45]. Browning of WAT is an adaptive and reversible response to numerous stimuli, including noradrenaline stimulation by cold exposure, exercise, natriuretic peptides, thyroid hormones, bile acids and nutritional compounds (resveratrol, menthol, capsaicin, etc.) [46, 47]. Other stimuli are due to pharmacological molecules, such as β3-adrenergic agonists, peroxisome proliferator-activated receptor γ (PPARγ) agonists thiazolidinediones and cannabinoid antagonist (rimonabant) [30, 48]. As a result of these stimuli, the transcriptional machinery of the browning program activates the expression of characteristic thermogenic genes (such as UCP1), leading to a beige adipocyte phenotype [30, 45, 47].

Two theories exist about the origin of beige adipocytes: (1) *de novo* differentiation from resident progenitor cells and (2) transdifferentiation. In detail, beige adipocytes can originate from progenitors resident within WAT that are differentiated in response to browning stimuli [39, 49–51] or, alternatively, they can arise via transdifferentiation, a process that involves the direct conversion of existing white adipocytes into brown-like fat cells [50]. This capacity of transdifferentiation is highly dependent on environment stimuli and also on the physiopathology aging [30, 52].

Beige adipocytes have a predominant lipid vacuole in the cytoplasm and numerous mitochondria, so exhibiting several intermediate features between white and brown adipocytes [33, 36, 47] (**Figure 1**), but these cells expressed characteristic and distinct gene markers that distinguished them from both white and brown fat cells [30, 39, 53, 54]. These genes encode proteins with very distinct cellular functions, including transcription factors (Zic1, Tbx15, etc.), metabolism-related proteins (Slc27a1, etc.) and proteins associated with inflammatory pathways

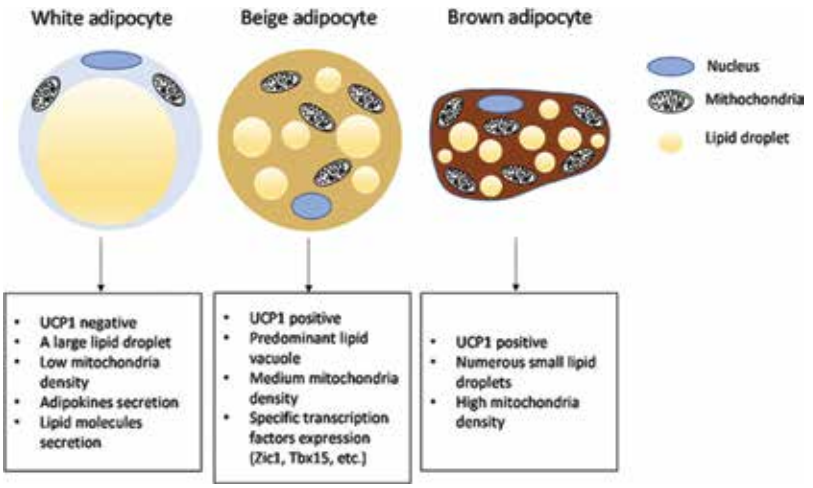

Figure 1. Main morphological characteristics of white, beige and brown adipocytes. UCP1: uncoupling protein 1.

(CD40, CD137, etc.) [35, 53–55]. It has been proved that more than 50 transcriptional molecules have been identified and their action mechanisms have been defined necessary in the browning transcriptional process [35, 56]. Among these, it is important to mention: PPARγ [50, 57], several members of the bone morphogenic protein family (BMP) [35, 58, 59], peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), which is involved in mitochondrial biogenesis [30, 33, 35] and also some transcription factors, such as C/EBPα and PRDM16 [58, 60, 61]. Even though the current terminology stresses the differences in cell lineage and localization, the evidence suggests that the beige adipocytes function as true thermogenic brown adipocytes [35, 47]. However, there is not a precise bioenergetic analysis of beige fat cells.

To date, the metabolic benefits of browning of WAT in humans remain to be fully established and the safety is a concern that must first be addressed regarding any method used to induce WAT browning. Cold exposure is a classic and efficient way to induce browning [62, 63], but its obvious discomfort, together with risks of hypothermia, makes it almost impractical for clinical use [50]. Therefore, browning agents, either endogenous or exogenous, provide an attractive alternative for limiting metabolic diseases.

Following paragraphs describe the potential SIRT1 involvement as a target molecule that can thereby contrast obesity, introducing briefly the effects of resveratrol as a SIRT1 inducer.

5. Sirtuins

SIRTs are NAD^+-dependent deacetylases present in all prokaryotic and eukaryotic cells (with the exception of several red *algae* and *archaea*) [64, 65]. Mammals possess seven isoforms, from 1 to 7, mainly known as anti-aging molecules [66–69]; however they are involved also in the regulation of numerous other cellular processes, such as integrity of chromatin [70, 71], cell cycle [72, 73], apoptosis [74, 75], energy metabolism [76–78], inflammation [79, 80] or detoxification [81, 82]. Their expression occurs throughout whole body differing in their cellular

localization (mitochondrion, cytoplasm or nucleus) and tissue distribution [81, 83–88] (**Table 1**). Biochemically, SIRTs are a class of proteins that posses mainly NAD^+-dependent lysine deacetylase activities [89, 90]; however, some particular isoforms, like SIRT4 or SIRT6, also hold ADP-ribosyl transfer activity [91, 92]. SIRT isoforms share a conserved core catalytic domain, a NAD^+-binding place, consists of two subdomains [93]. SIRT isoforms' structural differences are manifested at N- and C-terminal regions, which are variable among homologs and enable them to possess more than one type of catalytic activity [94].

So far, more than 30 SIRTs substrates were identified and, in general, are divided into two groups: histones and non-histone substrates and deacetylation of both is a quick response to stress stimuli or activators [95, 96]. SIRTs also serve as the regulators of transcription of genes in complex with other transcription factors [97].

Among the SIRT isoforms, the most attention has been focused on SIRT1, the ortholog of yeast Sir2 [64, 98]. During aging, its levels decrease [99] and this reduction occurs also during age-associated diseases (like neurodegenerative pathologies, cardiovascular diseases, metabolic syndrome, etc.) [100–102] making SIRT1 a possible treatment target. In fact, the beneficial effects of SIRT1 activation have been shown on numerous animal models [103–106] as well as humans [107–109]. Additionally, the positive effects of SIRT1 activation include also browning of WAT [110–112].

5.1. Sirtuin1 involvement in browning of white adipose tissue

All seven SIRT isoforms are expressed in adipose tissue [113–118], where they hydrolyse acetyl- and/or other acyl-group from the lysine residue of target substrate [119–121]. After particular stimuli, as summarized in **Figure 2**, the activation of SIRT1 at WAT level occurs and leads to the modulation (deacetylation) of PPARγ [110, 122], that with PRDM16 and PGC-1α, promote transcription of genes specific of BAT [61, 123].

Furthermore, PPARγ induces binding of C/EBPα and carboxy-terminal binding proteins 1 and 2 (CtBP 1 and 2) and represses transcription of genes which are specific of WAT [61, 124]. Thus, through PPARγ, SIRT1 is involved not only in enhancement of transcription of BAT genes, but also in repression of WAT genes. Furthermore, SIRT1 promotes mito-

Enzyme	Localization	Activity
SIRT1	Nucleus, cytoplasm	Deacetylase
SIRT2	Nucleus, cytoplasm	Deacetylase
SIRT3	Mitochondria, nucleus, Cytoplasm	Deacetylase
SIRT4	Mitochondria, cytoplasm	ADP-ribosyltransferase, lipoamidase, deacetylase
SIRT5	Mitochondria, cytoplasm, nucleus	Malonyl-, sukcinyl-, glutaryl-deacylase
SIRT6	Nucleus, endoplasmatic reticulum, cytoplasm	Deacetylase, ADP-ribozyltransferase, long-chain fatty acids deacylase
SIRT7	Nucleus (nucleolus), cytoplasm	Deacetylase

Table 1. Localization and catalytic activity of mammalian sirtuin isoforms.

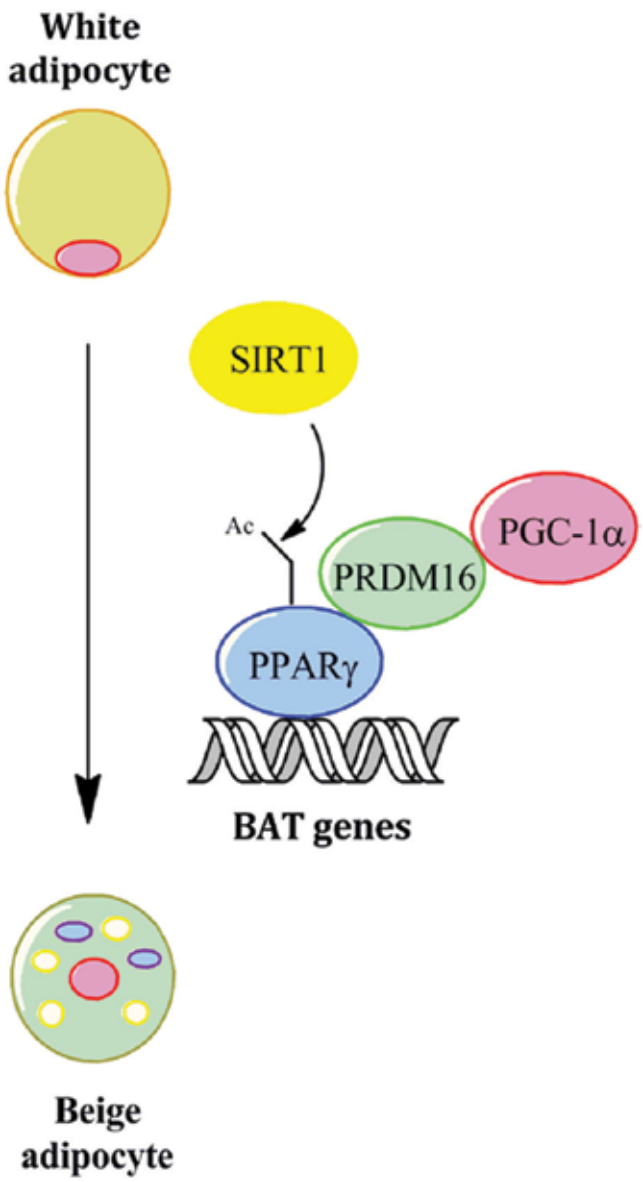

Figure 2. Involvement of sirtuin1 in browning process of white adipocyte. Sirtuin1 deacetylates PPARγ which than create a transcription complex with PRDM16 and PGC-1α promoting the beige adipocyte formation. SIRT1: sirtuin1; PGC-1α: peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PPARγ: peroxisome proliferator-activated receptor γ.

chondrial biogenesis by activating PGC-1α [125]. PGC-1α regulates also transcription of mitochondrial SIRT3, which is necessary for the full acquirement of BAT phenotype [117, 126]. In detail, PGC-1α promotes the transcription of SIRT3, which mediates the phosphorylation of CREB with subsequent enhanced expression of UCP1 and PGC-1α [117, 127]

(**Figure 3**). Furthermore, SIRT3 is involved in the regulation of many steps of mitochondrial metabolism, such as deacetylation of subunits of electron transport chain, for maintaining mitochondria proper functions [127–129].

Moreover, SIRT1 in adipose tissue might also decrease fat storage, promote lipolysis and protect against obesity-induced inflammation [130, 131]. Fang et al. [132] described a mechanism of regulation of SIRT1 activity by SIRT7, in detail, SIRT1 is able to augment its own catalytical activity by autodeacetylation and SIRT7 binds to SIRT1 and inhibits its activity. These data will help to clarify the mechanism of obesity in humans who showed decreased SIRT1 and increased SIRT7 in visceral adipose tissue in comparison to healthy normal-weight subjects [114]. However, Rappou

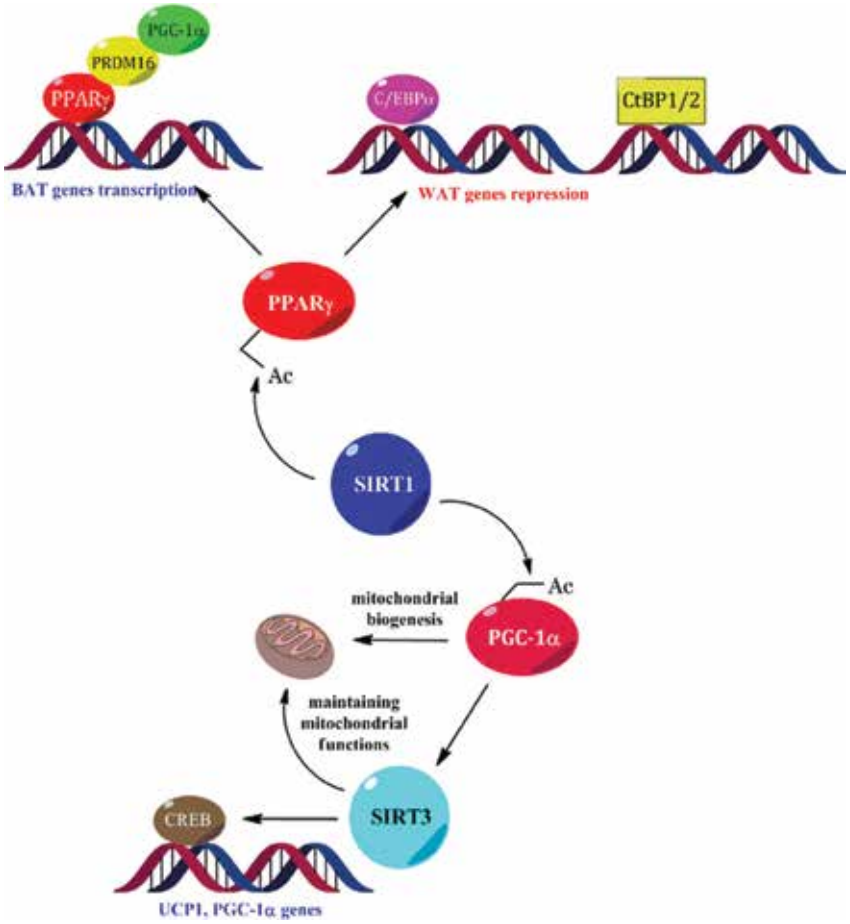

Figure 3. Sirtuin1 and sirtuin3 involvement in browning process. Sirtuin1 deacetylates PPARγ, which subsequently activates brown adipose tissue gene transcription and represses white adipose tissue genes. Sirtuin1 deacetylates also PGC-1α, which enhances mitochondrial biogenesis and sirtuin3 transcription, that is involved in maintaining mitochondrial functions and transcription of brown adipose tissue genes. CtBP 1/2: carboxy-terminal binding proteins 1 and 2; PGC-1α: peroxisome proliferator-activated receptor gamma coactivator-1 alpha; PPARγ: peroxisome proliferatoractivated receptor γ; SIRT1: sirtuin1; SIRT3: sirtuin3; UCP1: uncoupling protein1; WAT: white adipose tissue.

et al. [133] investigated the subcutaneous adipose tissue changes of all SIRT isoforms in obese subject with respect to normal-weight individuals and found decrease not only in SIRT1 and SIRT7 expression, but also in SIRT3. Moreover, they observed in the obese group that the continuous weight losers showed higher levels of SIRT1 in comparison to weight maintainers, suggesting that the individuals with naturally higher level of SIRT1 could be helped in weight loss.

Taken together, these data suggest that SIRTs, mainly SIRT1, could be strategic target in prevention and treatment of obesity and relative diseases. In fact, SIRTs activation results in many health benefits, including repression of adipogenesis [134] and promotion of browning of WAT [135, 136].

Polyphenolic compounds, among which the most studied in relation with SIRTs is resveratrol, might be dietary activators of SIRT1 [137–139]. Resveratrol is present in foods, like black and red grapes, blueberries, dark chocolate and peanuts [140–142]. In humans, its consumption protects low-density lipoprotein particles against oxidation promoting vascular health, decreases inflammation reducing C-reactive protein, tumor necrosis factor-α and interleukin-6 and also inhibits reactive oxygen species production [143–145]. Remarkably, resveratrol supplementation induced browning of WAT not only in rodents [146, 147], but also in human [135]. SIRT1 activation by resveratrol decreased PPARγ acetylation in 3T3-L1 white adipocytes and in human subcutaneous adipose tissue [110]. Furthermore, the overexpression of SIRT1 did not affect adipogenesis, but selectively decreased representative WAT genes [110]. This is in accordance with observations of Andrade et al. [146] who used resveratrol as SIRT1 activator in diet and showed attenuated expression of PPARγ and increased in UCP1 expression, contributing to loss of fat mass in comparison with mice fed without resveratrol.

It is interesting to cite also capsaicin as an inducer of WAT browning process through SIRT1 activation. The capsaicin, an irritant component of chili peppers [148], is not a direct activator of SIRT1, but it activates AMPK which, in turn, activates SIRT1 [149]. It may relieve neuropathic pain [150], osteoarthritis [151], migraine, headaches [152] or psoriasis [153] and it was studied also as a potent inducer of browning process [149, 154, 155]. Baskaran et al. [119] showed that the addition of 0.01% of capsaicin to high fat diet suppressed weight-gain in mice together with decrease in lipid content of epididymal and subcutaneous adipocytes. Furthermore, significant increase of SIRT1 and then of UCP1, PPARγ, PGC-1α and PRDM16 expression, promotes browning of WAT. However, it is difficult to study the effects of capsaicin in human due to low tolerance to capsaicin.

Resveratrol and capsaicin, together with other natural compounds, for example, green tea extracts, curcumin, melatonin or ω-3 polyunsaturated fatty acids [156, 157] and non-dietary inducers, such as endurance training and cold exposure [158], may represent interesting and promising stimuli of WAT browning process.

6. Conclusion

In summary, SIRT1 will be considered as an essential regulatory protein in browning of WAT. As BAT amount and SIRT1 expression decreased with age, targeted activation of SIRT1 is a promising strategy to stimulate browning of WAT. Activation of SIRT1 could be a novel

strategy in obesity treatment and related disorders. However, further studies are needed to better clarify the involvement of SIRT1 in the browning of WAT process and to identify more efficient SIRT1 inducers, which possess minimum side effects.

Conflict of interest

The Authors declare no conflict of interest.

Author details

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Chapter 5

Cellularity Description of Adipose Depots in Domesticated Animals

Olaia Urrutia, Leopoldo Alfonso and José A. Mendizabal

Additional information is available at the end of the chapter

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Abstract

Cellularity of adipose tissue in domesticated animals varies not only with species, sex, age and management conditions but also with depot. Differences in depots are important in animal production because of the economic and welfare implications and in humans in relation to obesity. The final amount of fat and its composition depends on the differentiation of mesenchymal multipotent precursor cells into mature adipocytes (adipogenesis) capable of fatty acid and triglyceride synthesis (lipogenesis), both processes being regulated by different key adipogenic and lipogenic genes, some of are well known and have been described. Histologically, differences can be classified as hyperplasia (an increase in adipocyte number) and hypertrophy (an increase in adipocyte size), processes that can produce adipocyte size distributions that are not necessarily Gaussian. A detailed description of the type of adipocyte size distribution can help distinguish the different adipocyte populations within depots and characterise each not only in terms of the size but also the number of the constituting cells. This description can help better understand the development and role of the different depots. It can also help when analysing causal relationships with adipogenic drivers and lipogenic enzymes involved in lipid metabolism.

Keywords: adipocyte, adipogenesis, lipogenesis, hyperplasia, hypertrophy, size, number, distribution

1. Introduction: cellularity of adipose tissue

Adipose tissue is a specialised connective tissue mesenchymal in origin formed by an association of cells called adipocytes, namely, cells that specialise in accumulating lipids. Adipose

tissue plays a fundamental role in maintaining the energy balance in animals, which entails storing energy in the form of fat (triglycerides) during periods of high energy intake and releasing energy through lipolysis during periods of caloric restriction [1]. However, certain discoveries made more than 20 years ago revealed that it is also a highly active endocrine tissue that secretes important substances, namely, adipokines and adipocytokines (*leptin*, *adiponectin*, *tumour necrosis factor alpha* or *TNF-α*, etc.), enabling it to communicate with different organs and demonstrating its involvement in such biological processes as neuroendocrine function and immune function [2, 3].

There are two main types of adipose tissue, white adipose tissue and brown adipose tissue, formed mainly of white and brown adipocytes, respectively. These tissues are differentiated not just only in terms of their colour but also in terms of their morphology, partitioning, genes and function [4]. The third adipocyte type known as "beige/brite" adipocytes growing in white adipose tissue in response to hormonal stimuli and cold has recently been described in rats and humans [5], as well as in cattle [6] and sheep [7].

Brown adipose tissue consists of small adipocytes (25–40 μm in diameter), which are multilocular, i.e., they contain several lipid droplets surrounded by large numbers of mitochondria; their main functions are thermogenesis, i.e., heat production, and thermal homeostasis [8]. This type of adipose tissue is typical in newborn animals in most species and in adult hibernating mammals [9, 10].

White adipose tissue is the predominant form in adult animals and is the main focus of the considerations set out here. White adipose tissue is composed mainly of adipocytes and a stromal vascular fraction comprising macrophages, capillary endothelial cells, undifferentiated preadipocytes, pluripotent stem cells and fibroblasts. This stroma is responsible for tissue homeostasis [11]. The adipocytes in white adipose tissue are ordinarily large (in cattle they can reach sizes of up to 180 μm in diameter) [12], and they have few mitochondria, their cytoplasm being almost entirely taken up by a single fat vacuole [13, 14]. In addition to its endocrine function, this fatty tissue subtype also plays a structural role, providing mechanical support and protection for certain parts of the body [3, 15, 16].

2. Adipose depots in domesticated animals

Adipose tissue is located in specific deposits, or depots, that are similar in mammals, though the size and composition of the depots vary with species, age, sex, diet, etc. [17]. The main depots are the visceral depots, comprising the kidney knob and channel fat (covering the kidneys and the pelvic cavity), mesenteric fat (surrounding the bowel) and omental fat (surrounding the pre-stomachs), the subcutaneous (SC) depot (covering the outside of the carcass), the intermuscular depot (located in between the muscles) and the intramuscular (IM) depot (infiltrated within the muscles) [18]. For example, Joy et al. [19] observed that omental fat accounted for 10%, kidney knob and channel fat 8.13%, mesenteric fat 8.01% and SC fat 25.3%, with intermuscular fat accounting for 42.6%, in lambs with a live weight of 23 kg fed a concentrate diet. Therefore, the importance of the IM depot is small compared with that of the other depots in quantitative terms.

The relevance of fatty tissue is the effect of the proportions and compositions of the different depots on production efficiency, nutritional value and meat and carcass quality. Fat is present in meat in the form of intermuscular fat, membrane phospholipids and IM and SC fat [20]. From a production standpoint, not all the depots have the same value, with the depositing of IM fat, or marbling, being desirable because of its beneficial effects on sensory attributes and hence its decisive role in determining meat quality [21]. In contrast, the accumulation of excess fat in certain depots has an adverse effect by increasing production costs while decreasing product quality [15, 22]. For instance, visceral fat is mostly discarded at slaughter, while an excess of SC fat lowers the commercial value of carcasses. Subcutaneous fat is also of concern to consumers, because excess SC fat and a high saturated fatty acid (SFA) content are viewed as detrimental to human health [15, 23].

Therefore, the current trend is towards producing lean meats with optimum fatness levels, in particular suitable amounts of IM fat. Additionally, a certain amount of covering, or subcutaneous, fat is necessary to reduce carcass moisture loss and keep the carcass from drying out while also guarding it against potential bacterial contamination [24].

Intramuscular fat composition is especially important because of its impact on meat quality from the standpoint of human health. IM fat is formed mainly by phospholipids, which have a high polyunsaturated fatty acid (PUFA) content, and by triglycerides, consisting mostly of SFAs and monounsaturated fatty acids (MUFAs) [25]. The phospholipid content in the muscle is relatively constant because of phospholipid's role as structural constituents of the cells, so it is not directly related to fatness. What is more, it is not particularly influenced by species, breed, diet or age, though the n−6/n−3 PUFA ratio may experience some variation according to the diet [26]. Unlike phospholipids, triglycerides in IM tissue may vary considerably from 0.2 to 5%, depending on accumulated fat levels, muscle location and breed [27]. Triglycerides make up most of the lipids (around 90%) in SC adipose tissue, and the PUFA content, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is low because phospholipids make up just a small proportion [28–30].

As already indicated, PUFAs are selectively deposited among the phospholipids associated with muscle cell membrane, which may furnish opportunities for modifying muscle PUFA content in ways beneficial to human health without increasing the amount of IM fat [20, 31].

3. Adipogenesis and lipogenesis

Adipogenesis is the process of differentiation by which multipotent mesenchymal precursor cells turn into mature adipose cells [32, 33]. Mesenchymal stem cells are fibroblast cells mesodermal in the origin capable of acting as precursors not only for adipose tissue but also for muscle, bone, cartilage and tendon [34]. There are two stages in the process of adipocyte differentiation: determination and terminal differentiation (**Figure 1**).

The first stage encompasses the mechanisms, whereby the pluripotent stem cell takes on the characteristics of the adipocyte lineage and involves transformation of the stem cell into a preadipocyte that is morphologically still the same as the precursor. During this transition, the

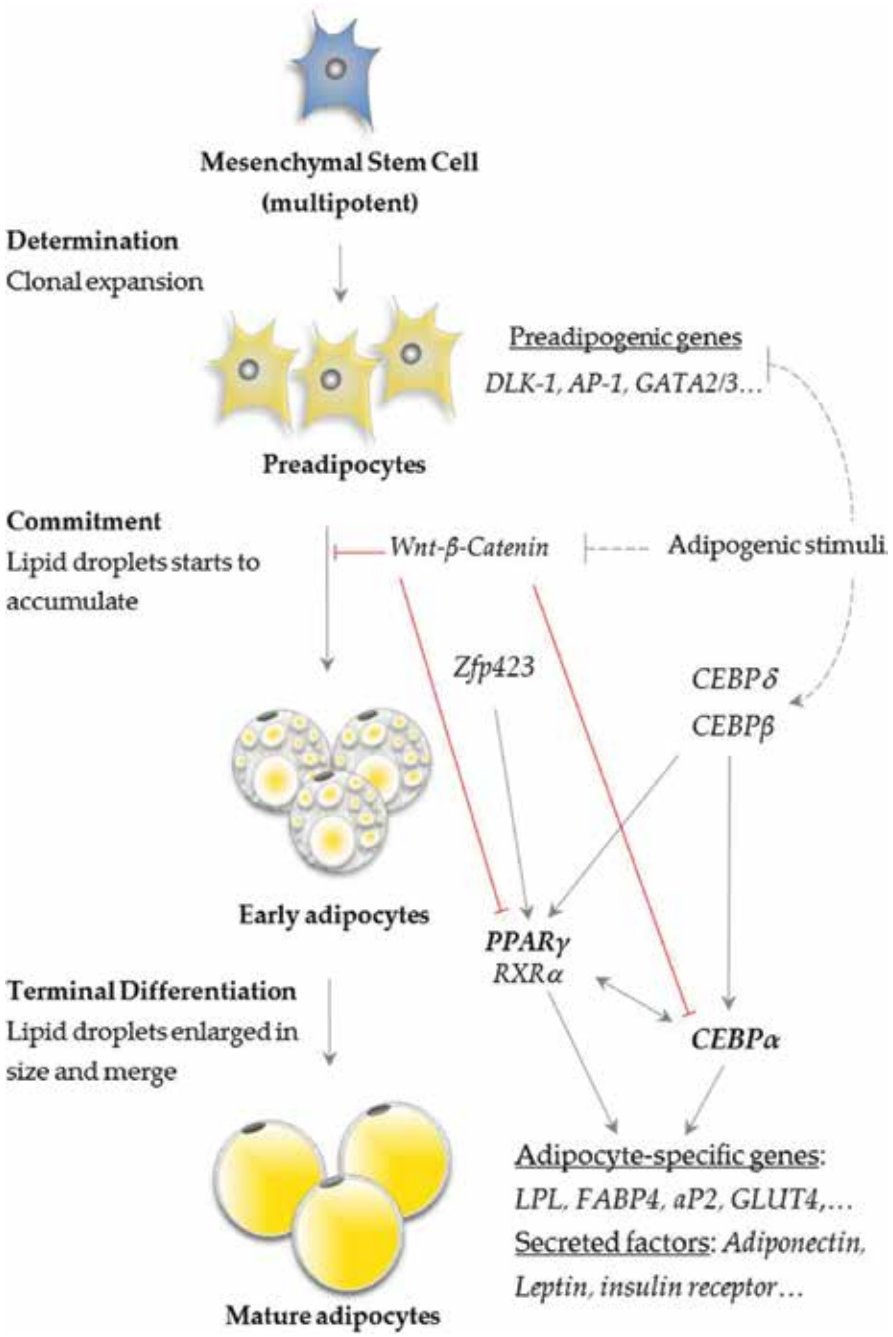

Figure 1. Schematic representation of the transition process from mesenchymal stem cell to mature adipocyte and transcriptional cascade during adipogenesis. *DLK-1 = Delta-like 1 homolog; AP-1 = activator protein-1; GATA2* and *3 = GATA binding proteins 2* and *3; WNT = wingless-type MMTV integration site family members; Zfp423 = zinc finger protein 423; CEBP* a, *β* and *δ = CCAAT/enhancer-binding protein* a, *β* and *δ; PPARg = peroxisome proliferator-activated receptor gamma; RXRa = retinoic X receptor; LPL = lipoprotein lipase; FABP4 = fatty acid-binding protein; aP2 = adipocyte protein 2* and *GLUT4 = glucose transporter type-4.*

cell loses its ability to turn into other types of cells [35]. Both positive (*zinc finger protein 423* or *Zfp423*; *activator protein-1* or *AP-1*) and negative (*delta-like 1 homologue* or *DLK1*, *GATA-binding proteins 2* and *3* and *wingless-type MMTV integration site family members* or *WNT*s) regulatory factors are known to take part in the initial stage, which is still poorly understood [36].

In the second stage of terminal differentiation, the preadipocytes acquire the complement of proteins needed for lipid transport and synthesis, insulin sensitivity and the ability to secrete adipokines and turn into mature adipocytes [9, 37]. This is accompanied by the accumulation of fat inside the cell and a change in morphology into a more globular shape [36]. This second stage is viewed as a cascade of transcriptional events in which the first wave consists of induction of *CCAAT/enhancer-binding protein (CEBP) β* (*CEBPβ*) and *δ* (*CEBPδ*), which activate expression of the central adipogenesis factors *peroxisome proliferator-activated receptor gamma* (*PPARγ)* and *CEBPα*. These transcription factors stimulate expression of genes involved in lipogenesis, such as *lipoprotein lipase* (*LPL*), *fatty acid-binding protein* (*FABP4)* or *adipocyte protein 2* (*aP2*) and *glucose transporter type-4* (*GLUT4*) [38, 39].

A variety of extracellular factors are capable of acting on the regulation of the above-mentioned transcription factors and are able to determine whether preadipocytes start the process of differentiation or remain quiescent. The activating factors include insulin, glucocorticoids, mono- and polyunsaturated fatty acids (which appear to be *PPARγ* activators) and prostaglandins [40].

Lipogenesis comprises the processes of fatty acid synthesis and triglyceride formation and takes place primarily in the liver, the white adipose tissue, and in the case of lactating females, the mammary gland. The biochemical mechanisms occurring in these tissues are similar, but the role of each of these processes varies by animal species. Adipose tissue is the main location of fatty acid synthesis in ruminants, and indeed in sheep, this tissue is responsible for over 90% of fatty acid (FA) biosynthesis [41]. As in ruminants, adipose tissue is the main site of fatty acid synthesis in pigs [42]. By contrast, in poultry it is the liver that is most active in de novo lipogenesis, while in rats, mice and rabbits, activity levels are similar in both tissues.

The fatty acids thus formed may be used to make cell membrane-building phospholipids, they may be used as precursors for biologically active metabolites or they may be used in the synthesis of triglycerides to store metabolic energy. This last-mentioned process is carried out by the endoplasmic reticulum in adipocytes and involves esterification of activated fatty acids (acyl-CoA molecules) through the action of acyl-CoA synthetase (ACS) and glycerol-3-phosphate from glucose catabolism.

Fatty acids from de novo synthesis are, as mentioned above, a source of acyl-CoA, but fatty acids ingested in the diet imported by *LPL* or released by lipolysis may also be used. Glycerol-3-phosphate is obtained by the action of the G3PDH enzyme on dihydroxyacetone phosphate (DHAP), though a small quantity may be produced by the action of glycerol kinase on glycerol [43].

4. Adipocyte hyperplasia and hypertrophy

Adipose tissue plasticity is high, and growth is produced by a combination of two mechanisms: hyperplasia, or an increase in the number of cells, and hypertrophy, or an increase in

cell volume. Hyperplasia is a proliferation of preadipocytes and their subsequent differentiation and occurs primarily in the animal foetus and postnatal period. Hypertrophy also takes place during this period, whereby adipocyte volume increases through the accumulation of lipids in the form of triglycerides, formed by the esterification of glycerol-3-phosphate, which is synthesised from glucose and fatty acids (from the diet or synthesised de novo) [4, 13, 44, 45].

After birth and the postnatal period, adipose tissue growth is mainly by hypertrophy of existing adipocytes and the activity of lipogenic enzymes [46]. Nevertheless, adipose tissue size in animals is not necessarily limited by the hyperplasia taking place at an early age, inasmuch as adipocytes can stimulate adipogenesis when a given percentage of adipocytes have reached their maximum volume, thereby inducing an increase in hyperplasia and/or promoting lipid accumulation by preadipocytes which had been quiescent until then [13, 43, 47].

Both hyperplasia and hypertrophy can be influenced by factors such as sex, breed, age, physical condition, diet type and amount, anatomical location of the adipose depots, etc. [48]. As a whole, fat deposition increases with animal weight and age, though development of the different fat depots is not uniform from either a quantitative or a temporal standpoint [49]. Generally speaking, the kidney knob and channel fat depot are the first to grow, followed by the intermuscular and SC depots and, lastly, the IM depot [13, 15]. In sheep, hyperplasia in the kidney knob and channel fat depot is complete approximately 60 days after birth, and further growth is ascribed to hypertrophy. Both processes contribute to growth of the intermuscular depot, with hyperplasia occurring until around 100 days after conception. In contrast, hyperplasia and hypertrophy together may contribute to SC depot growth until lambs turn 12 months of age [13, 44].

IM fat is a late-developing depot, and fat deposition in the IM depot would appear to depend mainly on hyperplasia [23, 50, 51]. In older animals the rate of fat accumulation outpaces muscle growth, in contrast to the situation at younger ages, and as a result, intramuscular fat deposition necessarily takes place later in life [23].

As already mentioned, adipose depots do not all grow at the same time or at the same rate, and adipocyte size therefore varies from one depot to another [22]. As a general rule, mean adipocyte size by depot in sheep [50, 52, 53] and cattle [54–57] follows this order: omental > kidney knob and channel fat > SC > intermuscular > IM. Furthermore, lipogenic activity in the different fat depots also varies, and this would appear to be related to adipocyte size and thus for the most part follows a similar order [22].

Intramuscular adipose tissue makes up a single depot, because it can be distinguished from other fat depots by its location within the perimysial connective tissue next to the myofibrils, and in addition its metabolism displays certain differences as compared to the other depots. Rates of fatty acid synthesis by intramuscular adipocytes are some 5–10% of the rate observed in the SC depot, which means that the fatty acid synthesising ability of the IM depot is low, as is its ability to break down fatty acids [58]. Gondret et al. [59] carried out proteomic analysis and reported that various lipogenesis, lipolysis, fatty acid oxidation and basal metabolic rate indicators were lower in IM tissue than in SC tissue.

Furthermore, intramuscular adipocytes chiefly use glucose and/or lactate as a carbon source for lipogenesis, whereas SC adipocytes use acetate [60]. Consequently, as Hausman et al. noted [58],

there is evidence that the IM and SC tissues are metabolically distinct, with these differences manifesting at the cellular level (preadipocytes and adipocytes), such that, for instance, the proliferation and differentiation potency of bovine intramuscular preadipocytes is lower than that of bovine subcutaneous preadipocytes [61, 62].

5. Adipocyte size distribution

Adipocyte size in domesticated animal populations has been estimated for decades [63]. At the present time, there are different methods of evaluating adipocyte size, by means of electronic measurement following fixation with osmium tetroxide, by means of histological analysis and by means of collagenase digestion. These last two methods are constrained as to the numbers of adipocytes that can be analysed per sample (around 250), but they are less expensive and require less expenditure on equipment [64]. The collagenase digestion method, in which collagenase is used to dissolve the connective tissue surrounding the adipocytes [65], is frequently used in animal studies [48, 66–69]. Succinctly, samples taken from animals at slaughter are stored in Tyrode's solution at 39°C, blood vessels and connective tissue are removed and the samples are washed in saline solution and digested using collagenase under incubation at 39°C for 1 and a half hours. The solution is then filtered through an 850 μm filter, microscope slides are prepared and the diameters of a sample of at least 200 of the adipocytes thus obtained are measured using image analysis.

Irrespective of the method employed, for many years now, the size distributions of adipocytes in different animal species have sometimes been observed to fit a normal distribution [48, 53, 70], but this is not always the case. Bimodal distributions have been described [55, 71–73], and this may, though not always [74], indicate the presence of a heterogeneous cell population [75]. The bimodal distribution of adipocyte size could thus be an indication that both hyperplasia and hypertrophy are taking place at the same time or that factors contributing to heterogeneity during adipocyte hypertrophy are at work. Growth in adipocyte size may increase local hypoxia, resulting in cell death as well as, after a certain critical volume has been reached, in the secretion of new adipocyte recruitment factors [76]. Another possible source of the bimodal distributions could be that the metabolic activity of adipocytes depends on individual cell surface size, since triglyceride exchange with the extracellular milieu occurs across the surface, and consequently differences in lipolytic and lipogenic fluxes could give rise to adipocyte populations that differ in size without hyperplasia necessarily taking place [77].

In any case, proper description of adipocyte size distributions is needed to be able to identify the factors underlying the differences in adipose tissue growth between groups of animals. If a distribution is bimodal, describing adipocyte size on the basis of the mean and the standard error of the mean is not meaningful, and using the mean to compare differences in experimental treatments could lead to erroneous conclusions. Compared with simply calculating mean cell size and the total number of adipocytes, studies that deal with adipocyte distributions are better at explaining the mechanisms involved in fat development [78]. For this reason, various approaches to evaluating adipocyte size distributions have been put forward, e.g., lognormal distribution fitting [72], normal distribution mixtures [79] and combinations of exponential

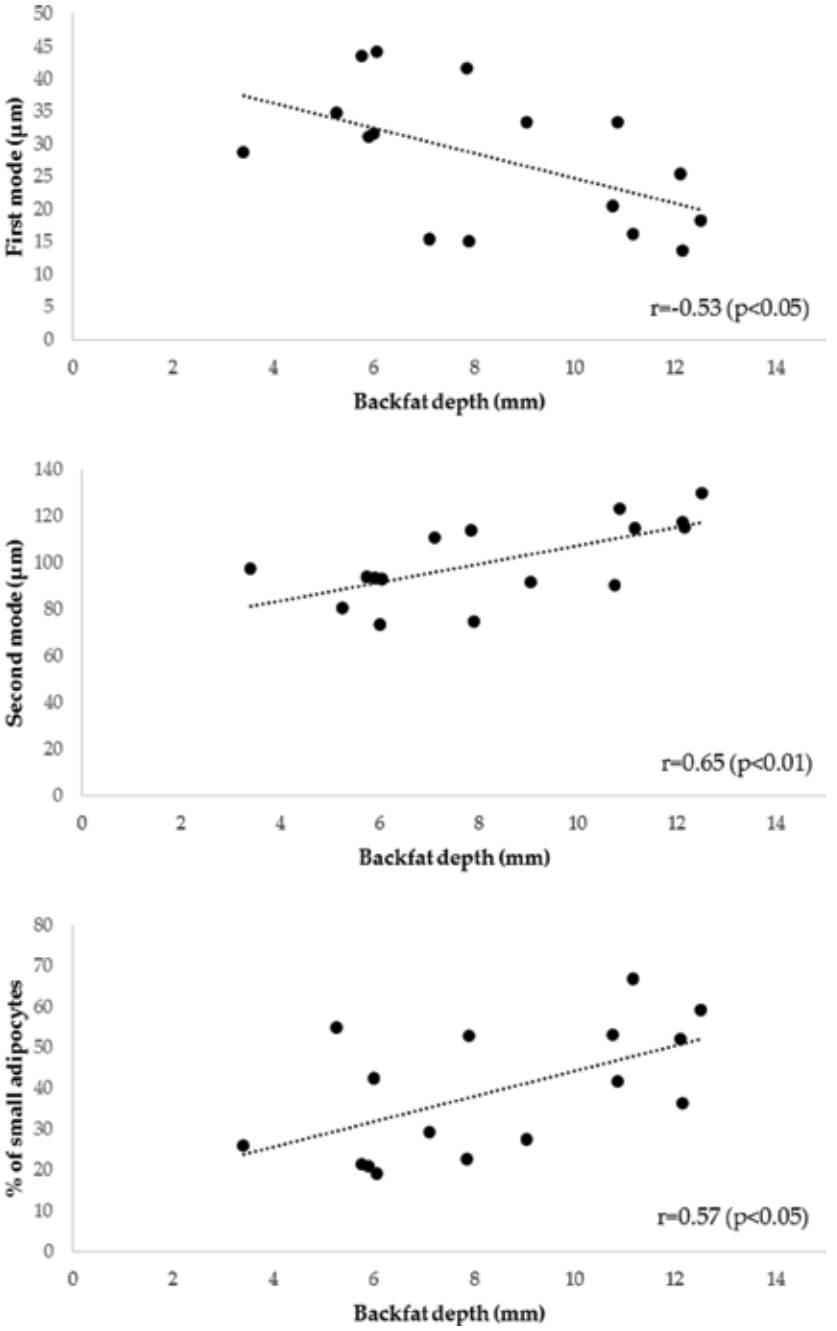

Figure 2. Relationship between the first mode, second mode and percentage small adipocytes and backfat thickness in the outer subcutaneous layer in brood sows (data after Abadía et al. [67]).

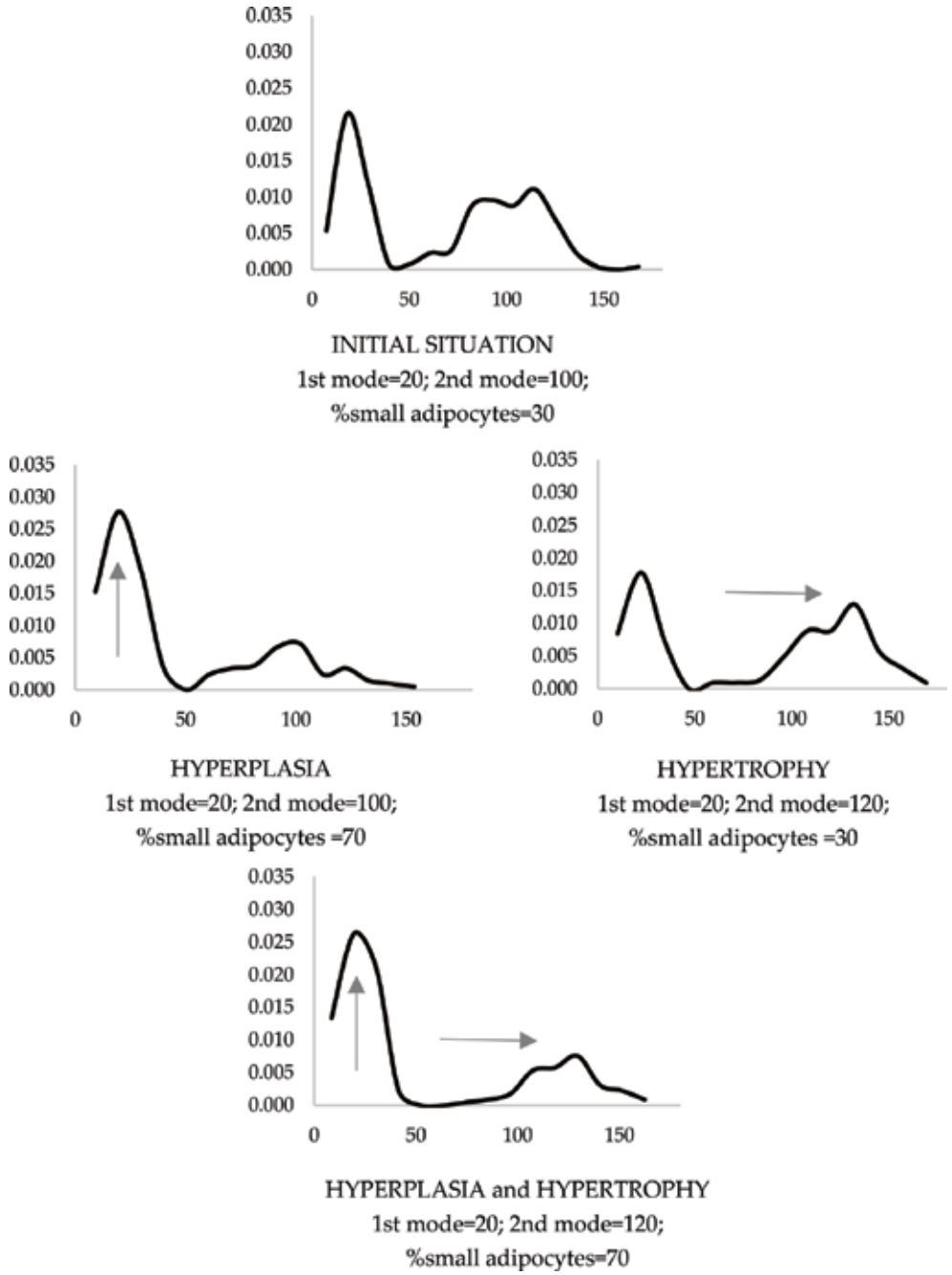

Figure 3. Simulated adipocyte size distribution depicting changes in the bimodal distributions in conditions of hyperplasia and hypertrophy. Arrows indicate the changes observed.

and normal distributions [80]. In any case, before putting any of these approaches to use, it is advisable first to check whether distributions are bimodal [81], since even exploratory data analysis methods like histograms may be misleading [82].

Alfonso and Mendizabal [81] proposed using the bimodality coefficient (BC) [83] and the dip statistic [84] to test the unimodal distribution of the data without having to assume any distribution underlying the bimodal distribution, though other approaches have also been described [85]. If the data prove not to be unimodal, the bimodal adipocyte size distribution can be described by estimating the two distribution modes and the proportion of adipocytes located under the inflexion point (nadir) between the two, thus enabling one population of small adipocytes to be differentiated from a second population of large ones. These three parameter values can be related to the degree of fatness in the animals as illustrated in **Figure 2**. The relationship needs not be unique but may vary with the species, tissue, age, physical growth, reserve mobilisation, etc. according to the relative importance of hyperplasia and hypertrophy that may be taking place at the time.

Figure 3 depicts what happens to these parameters with an increase in the number of small adipocytes (hyperplasia) or an increase in the size of large adipocytes (hypertrophy). This is a simulated example (as described by Alfonso [86]), which shows that the hypothesis of hyperplasia (an increase in the number of adipocytes) can be tested on the basis of the differences occurring in the percentage of small adipocytes and the hypothesis of hypertrophy (an increase in the diameter of large adipocytes) can be tested on the basis of the differences occurring in the second mode. The effect of both processes combined is also shown, since both changes in adipocyte number and changes in adipocyte size are known to take place simultaneously [87].

Analysing the data in this way allows differences between groups of animals to be described even in the presence of bimodality. Thus, differences between tissues in cattle [68] and pigs [81, 85], between breeds [85] and between animals grouped by age [88] and sex [85] have been described. Describing these differences is an essential basis for subsequently studying cell multiplication and differentiation both at the genetic level and at the level of expression, the better to explain the mechanisms of adipose tissue growth and be able to act appropriately so as to modulate them with a view to improving animal production systems. These same working methods can be extrapolated to the study of adipose tissue in humans and may help explain the factors involved in obesity and associated health issues.

6. Conclusion

Adipose tissue plays a fundamental role in maintaining the energy balance in animals, storing energy in the form of triglycerides during periods of high energy intake and releasing energy through lipolysis during periods of caloric restriction. In domesticated animals these triglycerides are located in different depots which vary in size and composition with species, sex, age and diet. These same factors in animal production systems condition adipose tissue growth brought about hyperplasia, or the proliferation of adipocytes, and hypertrophy, or the increase in adipocyte size. Both processes are regulated by different factors that stimulate or inhibit the expression of adipogenic and lipogenic genes. A knowledge of these processes enhances our

understanding of the genetic basis underlying the growth of fat depots in domesticated animals so as to be able to modify their quantity and composition. Thus, studying adipocyte size distributions helps us understand the different levels of involvement of, on the one hand, hypertrophy and hyperplasia and, on the other, of adipogenic and lipogenic genes and regulatory factors on adipose tissue growth in different groups of animals.

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Section 3

Adipose Tissue and Diseases

Chapter 6

Adipose Tissue and Inflammation

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Additional information is available at the end of the chapter

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Abstract

Adipose tissue is composed mainly by adipocytes and stromal-vascular fraction, which are composed by different cell types including macrophages. There are three types of adipose tissue: brown (BrAT), white (WAT), and beige (BeAT). BrAT is less abundant and is implicated in lipid oxidation and energy balance; BeAT has the pathway of adaptive thermogenesis, and WAT is endocrine in nature and lipid storage site and is implicated as an endocrine organ that secretes hormones and different molecules. These molecules are pro-inflammatory and anti-inflammatory factors, including the adipokines leptin, adiponectin, resistin, and visfatin, as well as cytokines and chemokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, leptin, adiponectin, and others, are involved with the development of adipose tissue inflammation and obesity. This pathological condition, together with other factors such as oxidative stress, may develop insulin resistance and the pathogenesis of type 2 *diabetes mellitus* (T2DM).

Keywords: adipose tissue, inflammation, oxidative stress, insulin resistance, type 2 *diabetes mellitus*

1. Introduction

Adipose tissue at first considered as a fat storage and insulation organ had a radical change when leptin, the hormone of obesity, was discovered [1]. Since then, the adipose tissue has gained more interest to know its role as an endocrine tissue and its capacity of producing

other molecules that have action in various parts of the body including metabolic regulation and the immune system. Adipose tissue is composed primarily by pre-adipocytes and adipocytes, but other types of cells called the stromal-vascular fraction composed by different cell types including macrophages, neutrophils, lymphocytes, fibroblast, and endothelial cells among others also are present [2]. The presence of these cells account for the vast secretion of molecules called adipokines with various significant physiological functions, many of them implicated in metabolism and others implicated in the balance between pro- and anti-inflammation to maintain the homeostasis. In obesity, there is a strong inflammatory response in adipose tissue, which is one of the most important causes for the development of insulin resistance and the pathogenesis of T2DM.

2. Morphology and physiology of adipose tissue

The adipose tissue originates from the mesenchymal cells and around the blood vessels in the 14th and 24th week of embryonic development. In the late stage, fibrous and vascular septa are formed between the aggregates of lipocytes that appear in the face, neck, breast, and abdominal wall at 14 weeks, and at 15 weeks, it is already evident in the back and the shoulder, later extending to limbs, higher and lower at 23 weeks. There is an excellent association between adipogenesis and angiogenesis, since lipoprotein-lipase accumulates in the lipocyte membrane, which transfers serum lipoproteins to lipocytes [3].

There are three types of adipose tissues: brown, white, and beige. They differ in development, anatomical location, and species. BrAT is less abundant and is implicated in lipid oxidation [2], and also has a great potential of impact on the energy balance. It is capable of rapidly producing large quantities of heat through the activation of the only uncoupling protein (UCP)-1 located in the mitochondria inner membrane. WAT is endocrine in nature and lipid storage site and is implicated as an endocrine organ that secretes hormones and different molecules as growth factors, complement factors, enzymes, and pro- and anti-inflammatory cytokines among others [2]; while BeAT is primarily white, it has some cells that have UCP-I. BrAT appears first in the fetus mid-gestation and is lost during the maturation of childhood, adolescence, and mature age [4].

2.1. White adipose tissue

Histology: the preadipocyte is spindle-shaped with 4–5 cell extensions and abundant rough endoplasmic reticulum (ER). Adipocyte of WAT is a spherical cell with variable size from 10 to 180 μm diameter depending of the content of the unique fat droplet (red arrow in **Figure 1A**) [5]; each fat cell is surrounded by its membrane, the center is occupied by a single-lipid vacuole, the cytoplasm is at the periphery as a rim, the nucleus is in flattened form and have a clear vacuole; in the rim of cytoplasm (arrowhead in **Figure 1B**), the tissue is subdivided into incomplete lobules by slim bands of collagen forming the stroma, in which there are several blood vessels (black arrow in **Figure 1A**), in the external membrane of endotelial cells of blood vessels are the lipoprotein lipase to translate free fatty acids to adipocyte increasing like this the vacuole of lipid [4].

http://dx.doi.org/10.5772/intechopen.74227

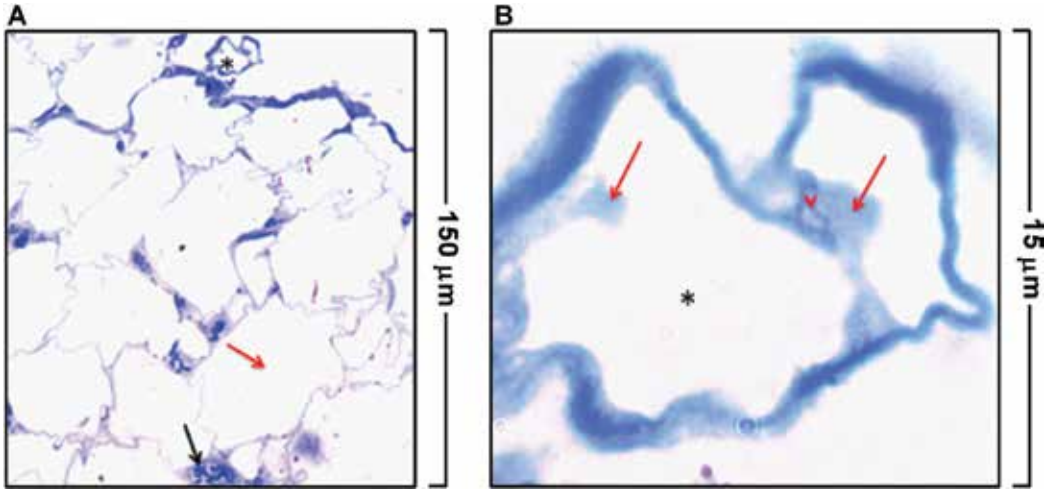

Figure 1. Photomicrography of white adipose tissue. (A) Photomicrography of WAT-150 μm. The red arrow shows the unilocular fat cell, cytoplasm. The black shows blood vessel. (B) Photomicrography of WAT-15 μm. The red arrows show the nucleus. V: blood vessel. Toluidine blue stain in L.R. White resin.

The capacity of storing fat during excess food intake enables the organism to use this fat as energy source in starvation condition or in significant energy expenditure. However, as mentioned above, the adipocytes also produce and secrete different molecules. Some of them act as hormones or like-hormones with actions related to the storage or utilization of fat, while other molecules produced by adipocytes play a role directly connected to the immune system in both innate and adaptive response. All these activities change the notion that adipocyte is merely a cell to store or release energy to a dynamic system of metabolic regulation as well as immune defense mechanism [6].

2.2. Brown adipose tissue

Histologically, BrAT is organized into lobules formed by fat cells surrounded by collagen bands mixed with capillaries, nerves, and connective tissues. In BrAT, in the cells, the lipid droplets accumulate in multiple vesicles and therefore appear as multivesicular (red arrow in **Figure 2A**) intermixed with abundant mitochondria, given to the cytoplasm a multigranular shape surrounding the prominent central nucleus (red arrow in **Figure 2B**). In some brown fat cells, the lipid gathers, then the droplets coalesce into a large vacuole that gives the cell first an oval and finally round shape as unilocular cell (asterisk in **Figure 2A**). The cells are polygonal in shape with a mixture of multivacuolated and univacuolated cells.

Brown adipose cells are surrounded by a network of collagen fibers that contain abundant minute sympathetic nerves and blood vessels. Non-myelinated axons terminate in fat cells, providing for a direct sympathetic regulation, and the vascularity is ample with numerous capillaries coursing between the adipocytes [4]. Brown adipose cells have numerous mitochondria that participate in heat production through UCP-1 protein. This protein is located in the inner mitochondrial membrane where it is responsible for uncoupling of adenosine-5′-triphosphate (ATP) production to use the conductance of H+ gradient to produce heat [7].

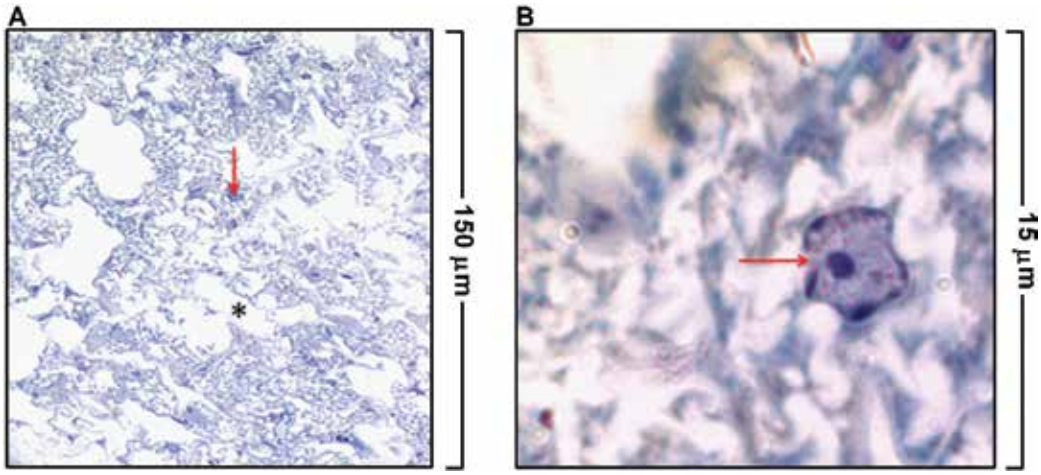

Figure 2. Photomicrography of Brown adipose tissue. (A) Photomicrography of BrAT-150 µm. The red arrow shows the multilocular fat cell and nucleus. The asterisk shows an unilocular fat cell. (B) Photomicrography of BrAT-15 µm. Red arrow shows the nucleus. Toluidine blue stain in L.R. White resin.

2.3. Beige adipose tissue

The BeAT is very similar histologically to BrAT; despite these similarities, it is now clear that the "classical" brown fat cells and the inducible "beige" fat cells come from different developmental lineages and are, in fact, distinct cell types; however, BeAT have the pathway of adaptive thermogenesis which is activated by cold via, and indirect pathway mediated by the sympathetic nervous system. Additionally, fat cells are also able to directly sense cold. The thermogenesis that is carried out in the Beige tissue is also by the UCP-1 protein. As mentioned above, this protein decouples the protons that would be used in the respiratory chain, thus transporting to heat producing in the inner membrane of the mitochondria instead of producing ATP; in this way, the chemical energy of the lipids and carbohydrates is released as heat and not by the formation of ATP [8].

This form of heat formation by adipocytes contributes to metabolic homeostasis, since the partial elimination of the UCP-1 protein in transgenic strains of mice is more susceptible to obesity and diabetes. Likewise, the total absence of the UCP-1 protein considerably increases the weight and fat content, which is demonstrated in the total absent mouse model of the forkhead box protein (FOX)-C2; these mice were induced to diabetes and obesity with considerable increase in brown and beige fat [8].

2.4. Genetics

At birth, there is approximately 16% of adult fat, and it is proliferating until puberty; from this the fatty tissue does not increase in volume except in exceptional cases of overfeeding, or slightly decreases in cases of strict diets of food reduction. The differentiation of adipocytes is controlled by the CHOP gene, which encodes a protein CCAAT/enhancer protein (C/CEBP), which also controls the differentiation of fibroblasts to adipocytes [4].

2.5. Physiology

White fat serves as a thermal insulator and protector of underlying tissues, and is essential in the uptake, synthesis, and storage of lipids and release of fatty acids in response to various neuronal and hormonal stimuli. This function is regulated by the hormone adipocyte lipoprotein lipase (LPL) which is increased in obesity and decreases with periods of lack of nutrition (starvation) and in *diabetes*. Insulin inhibits hormone-sensitive lipase and therefore blocks the release of fatty acids. It also serves to promote the uptake of glucose by adipocytes which are the precursor of glycerol phosphate, which in turn is necessary for the synthesis of triglycerides. Adipocytes have alpha and beta-adrenergic receptors; B1 adrenoreceptor agonists stimulate lipolysis, while alpha2 inhibitors inhibit it, both work through the cAMP as the second messenger. There are bodily differences, in some women there is a predominance of alpha 2 receptors in fat tissue of the gluteal region; therefore, although they are thin in other parts of the body, this gluteal region does not undergo changes in the fat content; on the other hand, some other people have more LPL, and based on the above, we can see that different body regions have different amounts of LPL receptors and alpha and beta-adrenergic receptors, which allow different deposits of fat in men and women. Growth hormone, insulin, glucocorticoids, and estradiol-17β stimulate the synthesis of DNA in adipocytes of both men and women [3].

3. Molecules secreted by adipocytes and stromal-vascular fraction

Although TNF-α, a potent pro-inflammatory cytokine [9, 10] was the first cytokine discovered that is secreted by adipose tissue [11], the leptin was the first molecule with hormonal activity that is secreted by adipocytes. Since then, multiple molecules called adipokines have been discovered as factors that are secreted by adipocytes or by the stromal-vascular fraction, composed mainly by macrophages, neutrophils, lymphocytes, fibroblast, and endothelial cells. This knowledge gave evidence that the adipose tissue modules various parts of the body and it is now considered as an organ endocrine, paracrine, or autocrine secreting molecules with activities as hormones, grow factors, chemotactic molecule, enzymes, and pro- and anti-inflammatory factors [12, 13].

3.1. Hormones

Adipocytes are cells metabolically dynamic that secrete multiple hormones that regulate the homeostasis. Leptin, acylation stimulating protein (ASP), adiponectin, resistin, and visfatin are hormones produced and secreted by adipocytes. These hormones play a key role over regulatory functions of various mechanisms such as beta-oxidation, fatty acid synthesis, and energy metabolism [14, 15]. Leptin is a hormone that suppresses food intake, and the gene that encodes this hormone is called obesity gene. Adiponectin is implicated in the metabolism of glucose and fatty acids. Acylation stimulating protein is concerned with fat-store. Resistin is a hormone implicated in resistance of insulin, and visfatin is a hormone with implication in the utilization of glucose. However, these hormones also are implicated in inflammation process

and they can interact with cells of the innate and adaptive immune system modulating the production of cytokines from adipocytes and the stromal-vascular fraction [16, 17] (**Figure 3**).

3.2. Growth factors

Fibroblast growth factors (FGFs), insulin-like growth factor (IGF)-1, hepatocyte growth factor (HGF), nerve growth factor (NGF), vascular endothelial cell growth factor (VEGF), and transforming growth factor (TGF)-β are growth factors that also can be produced by adipose tissue, and they can induce the adipogenesis [18], glucose metabolism [19], angiogenesis [20, 21] and thermogenesis [22]. However, they can also be implicated in inflammation processes (**Figure 3**).

3.3. Cytokines

There are many cytokines implicated in inflammation and there are differences in cytokine type according the individual status. The cytokines can be classified as pro-inflammatory or anti-inflammatory, although depending on the condition, some cytokines overlap both pro- and anti-inflammatory function. Also, depending on the condition of the body diverse

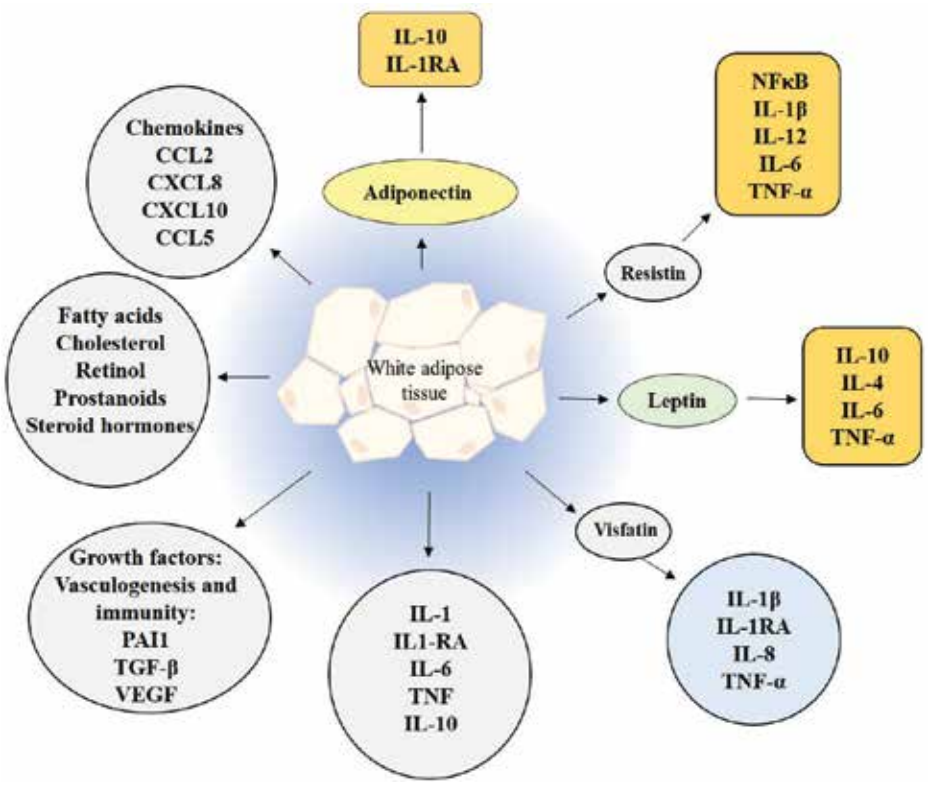

Figure 3. Adipokines secreted by adipose tissue linked to metabolic regulation and immune response. CCL, CC-chemokine ligand; CXCL, CXC-chemokine ligand; IL, interleukin; IL1-RA, interleukin-1-receptor antagonist; NGF, nerve growth factor; PAI-1, plasminogen activator inhibitor 1; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Description in the text.

types, cytokines can be produced; for example, in lean condition, IL-10, IL-4, IL-13, and IL-1 receptor antagonist (IL-Ra) are commonly produced and these cytokines exert anti-inflammatory function. On the contrary, obese individuals mainly produce and secrete IL-1, TNF-α, and IL-6 implicated in the inflammatory process [23, 24]. Although there are other cytokines produced by the adipocytes as Leukemia inhibitory factor, interferon (INF)-α, IL-6, and transforming growth factor (TGF)-β that can function as both pro-inflammatory and anti-inflammatory cytokines [25].

4. Relationship among adipokines and immune system

The relationship among adipokines with the immune response is complex. In addition to adipocytes, other cells are necessary in adipose tissue to exert its function. Cells like macrophages and lymphocytes can infiltrate the adipose tissue. There is a difference over the presence of these cells among individuals and the cell number is proportional to the size of the adipocytes. There is more infiltration of macrophages and lymphocytes in obese individual than normal individuals. Even more, there is a significant difference considering the base line number of these cells in normal individuals compared with the number observed in starvation and obese individuals [26, 27].

In the last 25 years, the relationship between adipose tissue and immune response has been extensively studied. The innate response with the production of pro-inflammatory cytokines is the main activity detected in adipocytes in normal condition. Macrophages are the main cell in adipose tissue that produces the first adipokines linking the innate and adaptive response. In normal condition, there is a homeostasis with different number of cytokines produced by macrophages mainly anti-inflammatory factors. In lean condition, macrophage population consist of M2 phenotype producing anti-inflammatory cytokine, whereas in obese condition, the macrophages are classically activated producing pro-inflammatory cytokine with increasing levels of TNF-α that exert multiples effects in different cells population around the body (**Figure 4**) [28].

The macrophages in adipose tissue have two origins. First, there is evidence that there exists a macrophage resident population established since embryonic development [29], and other population are monocyte-derived macrophages that respond to the inflammation process [30]. However, the population of macrophages that respond in the inflammation process is originated from circulating monocytes. In this initial process of inflammation, death adipocytes are capable to attract macrophages and depending of the type of cell death is the type of cytokine produced: apoptotic cells establish the production of anti-inflammatory cytokine, whereas necrotic cell establish a pro-inflammatory cytokine production characterized by the secretion of IL-1 from the macrophages population [31, 32]. Also, exogenous or endogen fatty acid as well as exogenous lipopolysaccharide are capable of inducing inflammation in adipose tissue through the activation of toll-like receptors present in both adipocytes and macrophages. However, the factors produced by the adipose tissue, both hormones and cytokines, have effect over other cell types including lymphocytes, dendritic cells, and neutrophils among others, affecting different mechanisms related with the production and release of multiple cytokines of the innate and adaptive response [16, 17, 33] (**Figure 4**). Now, the functionality

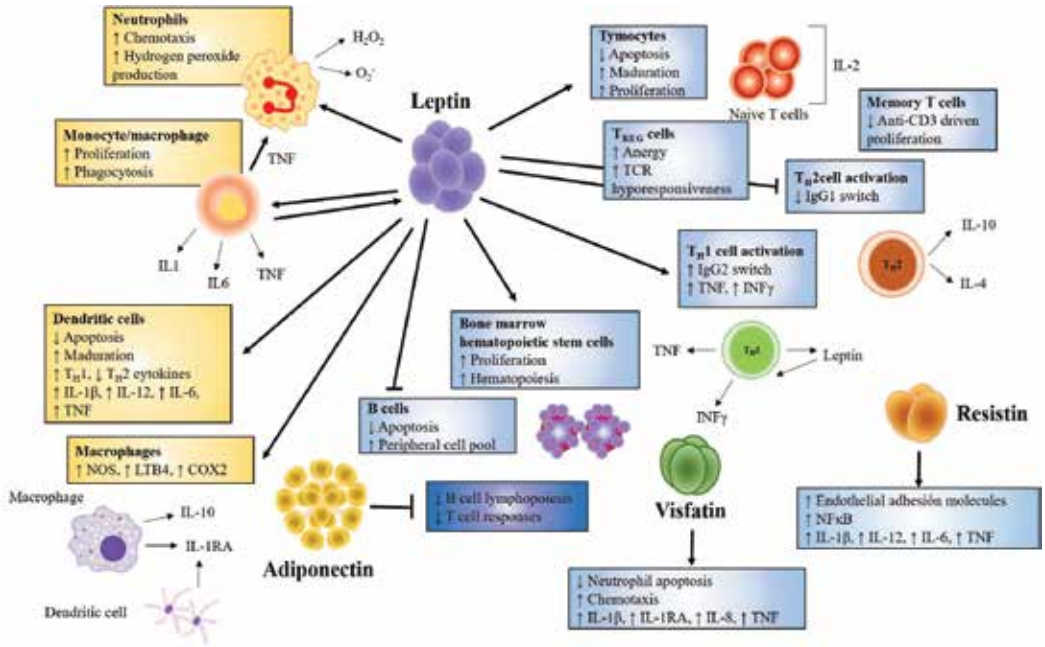

Figure 4. Adipokines and innate and adaptive response. Factors secreted by adipose tissue modulate various immunological process including activation and inactivation of leucocytes, and cell apoptosis, linking the innate and adaptive response. COX-2, cyclo-oxygenase 2; HMW, high molecular weight; IgG, immunoglobulin G; IFN, interferon; IL, interleukin; LTB-4, leukotriene B4; NFκB, nuclear factor κB; NOS, nitric oxide synthase; TCR, T-cell receptor; Th, helper T cell; TNF, tumor necrosis factor; T_{REG}, regulatory T cell. Description in the text.

of the adipose tissue under obesity condition have gained considerable interest mainly by its relationship with different diseases including *diabetes*, cardiovascular disease, metabolic syndrome, and its possible association with joint diseases [34, 35].

5. Relationship of obesity with inflammation, oxidative stress, insulin resistance, and type 2 *diabetes mellitus*

5.1. Obesity

Obesity is a medical condition with a major negative impact on health, mainly caused by a series of environmental, humoral, and genetic factors. Within these environmental factors such as the decrease in physical activity, sedentary lifestyle, and increase in foods with a high index [36], this produces a positive energy balance and this excess energy is stored in the form of fat that accumulates in the adipocyte and other organs such as the muscle and liver; however, some individuals are exposed to these factors and do not become obese, suggesting that genetic factors load may predispose individuals to develop obesity [37]. There are genes that have a high capacity to induce a state of obesity. Among them are those that regulate the intake and satiety, such as the gene of leptin or its receptor, alterations in the proopiomelanocortin

gene or in the melanocortin-4 receptor, mutations in some of these genes can cause the obesity [38]. On the other hand, investigations of recent years suggest the participation of inflammatory pathways and increased levels of pro-inflammatory cytokines, such as the nuclear factor (NF)-κB resulting in an increase of the IL-6, TNF-α, and IL-1β [39], with the development of obesity and obesity-associated insulin resistance, since inflammation caused a significant deterioration in the signaling pathways of insulin and leptin [39–42].

5.2. Inflammation

Inflammation is a process of activation of innate immune system, in response to exogenous and endogenous factors, such as infection by microorganisms, tissue stress, and injury [43]. The inflammatory response is characterized by its cardinal signs, such as redness, swelling, heat, pain, and interrupted function [44]. The inflammatory response consists of four main components: (1) endogenous or exogenous factors, such as molecular patterns associated with pathogens (PAMP) and damages (DAMP), which are derived from bacteria, viruses, fungi, parasites, and cell damage, as well as toxic cellular components or any other harmful condition [45]; (2) cellular receptors that recognize these molecular patterns (PRR), including Toll-like receptors (TLRs), nucleotide binding oligomerization domain (NOD)-like receptors (NLR), and retinoic acid inducible gene (RIG)-like receptors [46, 47]; (3) pro-inflammatory mediators, such as cytokines, chemokines, and the complement system [48]; (4) target cells and tissues, where these pro-inflammatory mediators act. The inflammatory response is characterized mainly by four successive phases: (1) silent phase, where the cells synthesize and release the first pro-inflammatory mediators, (2) vascular phase, characterized by increased vascular permeability and dilatation, (3) phase cellular, which is characterized by the infiltration of inflammatory cells to the site of damage, and (4) resolution of the inflammatory response [49–52].

Adipocytes and macrophages of the stromal fraction of adipose tissue express elevated levels of PRRs in response to various stimuli. The activation of PRRs activates a signaling cascade through canonical adapters of receptors, for example, the activation of TLRs that activates myeloid differentiation primary response (MYD)-88 located in plasmatic membrane or in endosomes associated with Toll IL-1r (TIR). This interaction activates a family of kinases which in turn activate different molecules including NFκB, a primary transcription factor. The translocation of NFκB to the nucleus upregulates the production of INF and inflammatory cytokines, which is an evidence that these receptors play a role in the immune system [53, 54].

5.3. Inflammation and obesity

The alteration of the metabolic syndrome as a consequence of obesity is one of the most common factors that evoke the activation of inflammation, producing other alterations such as oxidation, cellular hypertrophy, and stress, among others [55].

The metabolic and immune systems are regulated among themselves, and are made up of hormones, cytokines, signaling proteins, transcription factors, and bioactive lipids. The basic inflammatory response therefore promotes a catabolic state and suppresses anabolic pathways, such as the highly conserved and potent insulin signaling pathway [55, 56].

The inflammation attributed to obesity is given by the excessive intake of fats, macro nutrients, and foods rich in antioxidants causing deposition in other organs, mainly in the liver, altering insulin levels. There are other conditions due to obesity, such as alterations in blood pressure, heart rate, respiratory rate, and psychological factors [56, 57].

Obesity causes the increase and the extension of adipose tissue, and molecularly induces the release of signals and protein mediators called adipokines. The inflammatory response is derived from the high production of adipokines that produce the release of inflammatory mediators such as leptin, adiponectin, TNF-α, IL-1β, IL-6, protein monocyte chemotactic (MCP)-1, macrophage migration inhibitory factor (MIF), NGF, VEGF, plasminogen activator inhibitor (PAI)-1, and haptoglobin [39]. Several studies have shown the expression of these pro-inflammatory mediators (mainly TNF-α and IL-1β) in metabolic alterations [40].

The increase of evidence in studies of human population and research in animals has established causal links of diseases such as insulin resistance, T2DM, and metabolic syndrome as a result of the increase of adipocytes [40].

A study by Haiyan Xu et al. demonstrated the active role of macrophages in morbid obesity and the relationship they have with inflammatory processes, concluding that a disease of chronic inflammation initiated in adipose tissue is a consequence of insulin resistance [58, 59]. While it is clear that the inhibition of insulin receptor signaling pathways is a central mechanism by which inflammatory and stress responses mediate insulin resistance, it is likely that other pathways, molecules, and proteins have not been discovered yet with an alternative mechanism involved in this interaction [59, 60].

Recent research shows that the main risk to develop a metabolic complication is insulin resistance. However, in addition to cardiovascular and hematological diseases, pathologies such as fatty liver, airway diseases, cancer among other diseases that increase lipid or cytokine levels and, in turn, develop insulin resistance in the absence of obesity are related [61].

It is important to understand the inflammatory pathogenic mechanisms that produce diseases in the absence or presence of obesity. The aim is to reduce the rate of morbidity and mortality by preventing and treating pathologies that link inflammation with obesity [40].

5.4. Oxidative stress and obesity

Previous studies have reported and suggested that obesity and oxidative stress have relationship. Some markers of oxidative stress such as malondialdehyde (MDA) are increased in obese people by the production of reactive oxygen species (ROS) and for decrease in activity of antioxidant enzymes such as serum superoxide dismutase (SOD) and catalase (CAT). These factors are involved in the development of oxidation, cell damage, and metabolic diseases [62]. However, other studies suggest that obesity *per se* can induce systemic oxidative stress through multiple biochemical mechanisms, such as the generation of superoxide, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, oxidative phosphorylation, glyceraldehyde autoxidation, activation of protein kinase (PK)-C and pathways of hexosamine and polyol. On the other hand, studies suggest that obesity may be caused by oxidative stress through the

proliferation of preadipocytes and increased size of differentiated adipocytes [63–65]. Obesity is an important risk factor for the development of metabolic syndrome (MetS), *diabetes mellitus*, dyslipidemia, atherosclerosis, hypertension, insulin resistance, hepatic steatosis, non-alcoholic liver disease, and high morbidity and mortality [66] (**Figure 5**).

Several investigations and different clinical disciplines in recent years have focused their interest on oxidative stress. This evidence is associated with the pathogenesis of different diseases among which are *diabetes*, obesity, cancer, aging, inflammation, neurodegenerative disorders, hypertension, apoptosis, cardiovascular diseases, and heart failure [67], and is considered responsible for the beginning and progression of sundry brain disorders that include major depressive disorder [68]. From these investigations appears the concept that oxidative stress is the "common final pathway" through, whereby diseases produce their harmful effects. Oxidative stress causes complex alterations of cell metabolism and cell–cell homeostasis, in particular, oxidative stress is the key in the pathogenesis of insulin resistance and B cell dysfunction as well as in obesity [67].

Janus gas, oxygen (O_2), has positive beneficial effects as side effects injurious for biological systems. Its reactivity allows oxygen to participate in the transfer of high-energy electrons and therefore generates large amounts of ATP through oxidative phosphorylation. Just as oxygen is essential for life [66] and necessary to allow the evolution of multicellular organisms, it is also capable of attacking any biological macromolecule and the protein, lipid, or deoxyribonucleic acid (DNA) [66, 69], provoking diseases through an uncontrolled production of oxygen free radicals (RLO) and attacking cellular processes (enzymes production and cellular respiration) [66]. These events cause our body subdued to a constant oxidative attack of ROS [69]. Oxidation is the process that is held by the loss of electrons, which is always

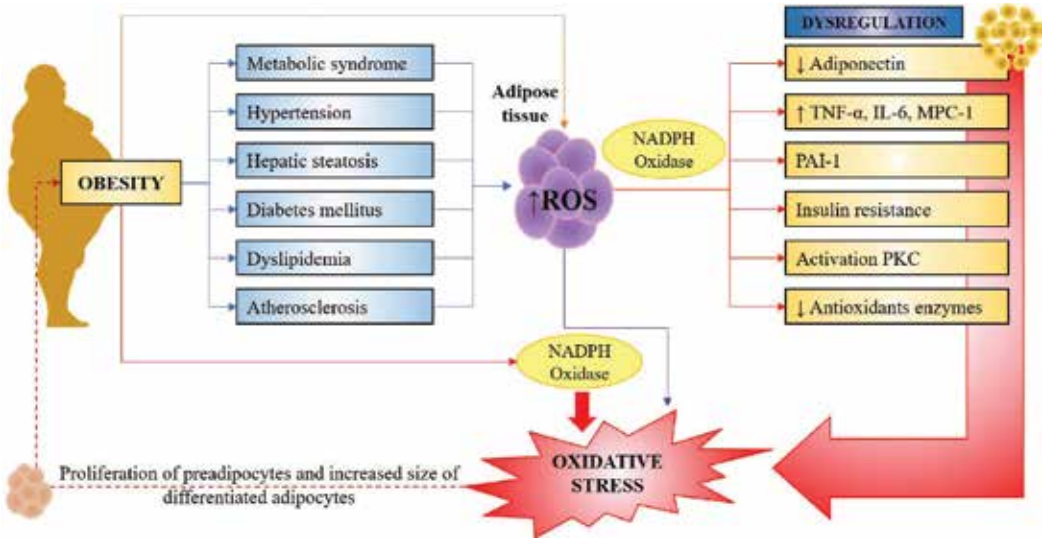

Figure 5. Factors contributing to increased oxidative stress (OS) in adipocytes and linked to insulin resistance, inflammation of adipose tissue, and decreased adiponectin. TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1. Description in the text.

associated with another catchment (reduction). This oxidation is essential for life and participates in processes of obtaining cellular energy [66]. The damage produced by the imbalance between oxidants and antioxidants in favor of oxidants is called "oxidative stress." Oxidants are formed as a normal product of aerobic metabolism, but its production increases rapidly under pathophysiological conditions [70].

Oxidative stress can be defined as the appearance of macromolecular damage from free radicals and thiol alterations that lead to redox control dysfunction [71], where the excess of endogenous oxidative species can damage the cells and manipulate the signaling pathways. Oxidative stress agents that occur at low physiological levels in mitochondria and peroxisomes are: reactive species, ROS such as superoxide, hydrogen, peroxide, and hydroxyl ion radicals. The endogenous ROS that occur at low levels are physiologically important, especially in signaling pathways, although these mechanisms are not yet clear because they perform a double function both as a signal and as a damaging agent. Among these signaling roles are transcriptional control and cell cycle regulation. Oxidative stress occurs when free radical species such as ROS and reactive nitrogen species exceed the capacity of cells to eliminate them through their antioxidant defense mechanisms, which have multiple nocuous effects on the cellular metabolism [65, 72]. It has long been accepted that ROS becomes harmful to cells even at low physiological levels. Recent studies have concluded that ROS damage has a direct role in the development and progression of many chronic diseases, including the pathogenesis of insulin resistance and T2DM [73].

Several studies have reported that obesity is associated with oxidative stress caused by an imbalance related to inadequate antioxidant defenses and an increase in the proportion of free radical formation [65, 74]. However, other studies suggest that obesity *per se* can induce systemic oxidative stress through multiple biochemical mechanisms, such as the generation of superoxide NADPH oxidase, oxidative phosphorylation, glyceraldehyde autoxidation, activation of PKC, and pathways of hexosamine and polyol. Hyperleptinemia, tissue dysfunction, low antioxidant defenses, chronic inflammation, and the generation of postprandial ROS are factors involved in the development of obesity in which oxidative stress participates [75] (**Figure 5**). As the increase in oxidative stress in accumulated fat is, at least in part, the underlying cause of adipocytokine dysregulation and the development of the metabolic syndrome [76], it is known that obesity induces oxidative stress through various mechanisms such as chronic inflammation, endothelial dysfunction, and mitochondrial dysfunction [77].

In vitro and in vivo studies suggest that obesity may be caused by oxidative stress through the proliferation of preadipocytes and increased size of differentiated adipocytes [63, 64]. The mass of adipose tissue increases when the pre-adipocytes differentiated in the terminal phase re-enter the cell cycle and undergo proliferation, process called adipogenesis, which includes the proliferation of preadipocytes and their differentiation into mature adipocytes [64]. It has been shown that ROS are implicated in both events. The proliferation of pre-adipocytes activated by ROS participates in the development of metabolic disorders, which generate more ROS through mechanisms that include chronic inflammation of adipocytes, oxidation of fatty acids, excessive oxygen consumption, and accumulation of cell damage, diet, and mitochondrial activity [64, 78]. Abnormal generation of ROS induces cellular dysregulation in many other tissues and promotes obesity [65].

The generation of ROS by the accumulation of adipocytes through the activation of NADPH oxidase produces dysregulation of the expression of inflammatory adipocytokines that include adiponectin, PAI-1, IL-6, and MCP-1 and decrease in the production of antioxidant enzymes [71] (**Figure 5**). Some biomarkers that are known for oxidative stress in serum and urine plasma are: MDA, isoprostanes F-2 (F2-IsoP), Prostaglandin F2a 8-iso (8-isoPGF2a), and carbonylation proteins. A significant positive correlation has been observed between the corporal mass index (BMI) and the biomarkers of oxidative stress [79]. Lower activity of antioxidant enzymes Cu-Zn superoxide dismutase (SOD) [75], Cat [62], and glutathione peroxidase (GPx) has been reported in erythrocytes of obese subjects than in non-obese controls [75, 80, 81].

Obesity is an important risk factor for the development of MetS, *diabetes mellitus*, dyslipidemia, atherosclerosis, hypertension, insulin resistance, hepatic steatosis, non-alcoholic liver disease, and high morbidity and mortality. Sundry mechanisms may be involved in the development of comorbidity related to obesity, including the abnormal production of adipocytokines, aberrant oxidative stress, and dysregulated pro-inflammatory response in tissues such as muscle and liver. It has been reported that both obesity and oxidative stress can occur in the first two decades of life [82] and accumulated fat is considered an early indicator of the metabolic syndrome associated with obesity which is related to a multitude of different diseases [74]. Therefore, when there is an excess in oxidation, oxidative stress appears which becomes complex at all biological levels since it is not possible to measure it or define it with a single parameter [67]. For all the above reasons, the development of new therapies should be considered as a major objective [76] for the treatment and control of oxidative stress in obesity as well as for diseases in which oxidative stress is involved.

5.5. Insulin resistance and obesity

Insulin exerts its effect through binding to its receptor; the insulin receptor (IR) has two isoforms: IR-A and IR-B. IR-A is found mainly in the adult nervous system and has a much higher affinity for insulin than IR-B, and this latter is mainly found in adipose tissue, liver tissue, and skeletal muscle [83]. This receptor is constituted by two α- and two β-subunits with a molecular weight of 350 kDa. The α-subunits are located on the outside of the plasma membrane and contain insulin-binding sites, while the β-subunits have a transmembrane, a juxtamembrane and an intracellular domain, where tyrosine kinase activity is found [84].

Under normal conditions, insulin binds to the α-subunit of the IR and promotes the autophosphorylation of the tyrosine residues of the β-subunit of the receptor. Then, there is a transphosphorylation that is recognized by some adapter molecules, among them are the insulin receptor substrates (IRS), that there are three types and are expressed in humans. IRS-1 and IRS-2 are widely distributed in mammalian tissue, while IRS-4 restricts its expression to the hypothalamus [85], these proteins are adapter molecules for the kinase activity of the phosphorylated insulin receptor, in turn phosphorylating the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K). The phosphorylation of the latter induces a conformational change of this protein which leads to the binding of the catalytic subunit (p110) activating PI3K; once activated, this protein phosphorylates phosphatidylinositol-3,4-diphosphate (PIP2) into the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3) [86, 87], and these lipid products induce

the recruitment and the interaction of the protein kinase (PDK)-1 and serine/threonine-specific protein kinase (Akt). The activation of PDK-1 phosphorylates some isoforms of protein kinase C, such as PKCλ/ζ, which is responsible for phosphorylating vesicles that contain the glucose transporter type 4 (GLUT4), promoting migration and fusion with the cell surface, which increases glucose uptake as well as your metabolism [88] (**Figure 6A**).

As we know, glucose is the main source of energy used by the organism and the only source of energy for the brain. Thus, glucose homeostasis must be finely regulated, and blood glucose levels must be kept within a very defined range (70–90 mg/dL). When the organism presents obesity, this regulation can be altered together with other factors, then insulin resistance (IR) appears [89]. This alteration is defined as a pathological state where there is a decrease in the metabolic response of target cells, tissue, or the whole organism to the action of insulin. Although acute hyperinsulinemia can be tolerated, chronic hyperinsulinemia exacerbates insulin resistance in different tissues and contributes to the failure of β cells to produce insulin and ultimately *diabetes* is established [90]. Insulin resistance is characterized by a series of alterations at the intracellular level, such as a decrease in the concentration of the receptor and its kinase activity, the concentration and phosphorylation of IRS-1 and IRS-2, PI3K activity, translocation to the membrane of the GLUT4 and the activity of intracellular enzymes [91]. Insulin resistance has been associated mainly with a state of obesity and physical inactivity, as well as a genetic predisposition, the stress on the β cells, causing an alteration in the function of these cells and a progressive decrease in the secretion of insulin [92]. IR can be divided into two types: hepatic IR or peripheral RI. Hepatic RI is an altered state of the synthesis of glucose in the liver, while peripheral RI is the reduced response of the skeletal muscle or adipose tissue to the action of insulin [93]. In the muscle, the alterations that contribute to develop RI can be mentioned as defects in: the insulin signaling, in the glucose transporter, in the phosphorylation of glucose, in the synthesis of glycogen, in the activity of the pyruvate dehydrogenase complex and in mitochondrial oxidative activity [94].

On the other hand, hepatic IR increases gluconeogenesis since some substrates (fatty acids, lactate, glycerol and amino acids) are derived from a deficiency of insulin signaling, hyperglucagonemia, and increased sensitivity to glucagon, which leads to increased gluconeogenesis; this overproduction of glucose by the liver occurs in the presence of increased levels of insulin, indicating resistance to insulin-induced suppression of hepatic glucose. This increase may have other factors that contribute to accelerate this production: increased glucagon levels and an increase in liver sensitivity to glucagon; lipotoxicity that induces an increase in the expression and activity of the enzymes phosphoenolpyruvate carboxykinase and pyruvate carboxylase; glucotoxicity that increases the expression and activity of glucose-6-phosphatase [95].

With the development of IR, the β cells of the pancreas increase the production and secretion of insulin as a compensatory mechanism (hyperinsulinemia) and the insulin receptor is insensitive to the action of this hormone, increasing the biochemical levels in blood as, glucose, triglycerides, and cholesterol, and lowering high density lipoprotein (HDL) cholesterol; this imbalance contributes to the development of cardiovascular diseases and metabolic syndrome [96]. The prolonged elevation of insulin at systemic level can lead to dysfunction in the signaling of the insulin receptor, mainly of the skeletal muscle, since when it is in a state of

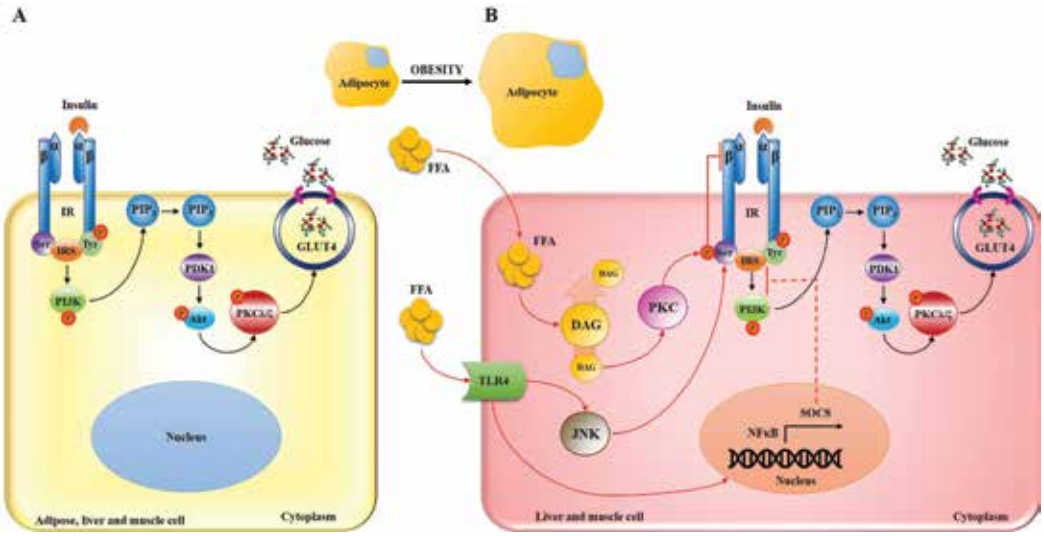

Figure 6. Insulin signaling in normal and obesity conditions. (A) Insulin signaling and glucose production under normal conditions. (B) Insulin resistance and obesity. IR, insulin receptor; IRS, insulin receptor substrates; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-3,4-diphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PDK-1, protein kinase 1; Akt, serine/threonine-specific protein kinase; GLUT4, glucose transporter type 4; FFA, free fatty acids; DAG, diacylglycerol; PKC, protein kinase C; JNK, C-Jun N-terminal kinases; TLR4, Toll-like receptor 4. Description in the text.

insulin resistance, the synthesis of glycogen is altered due to the decrease in glucose uptake. Insulin loses its ability to lower glucose levels through the transcription factor FOXO1 in the liver because the hormone loses its ability to inhibit this factor, which is involved in regulating gluconeogenesis enzymes. On the other hand, the disruption in the translocation of the GLUT4 glucose transporter from the cytoplasm to the membrane decreases its expression on the membrane, which results in a reduced uptake of circulating glucose.

Insulin resistance is associated with the phosphorylation of the IRS, since there is an increase in phosphorylation in the serine and tyrosine residues (serine/threonine) of this protein, which causes phosphorylation decrease and reduction in the interaction with the PI3K and the Akt, thus decreasing its activity. There is also evidence that adipose tissue plays an important role in the development of insulin resistance, and this tissue can regulate the metabolism of glucose in the body, through the regulation of free fatty acids, the secretion of adipokines, since it acts as an endocrine organ [97]. The ectopic accumulation of lipids in muscle and liver induces insulin resistance through the increase in the levels of diacylglycerol (DAG), and its accumulation promotes the translocation and activation of the protein kinase (PK)-C in the tissues, the PKC-θ in the muscle, and in the liver the PKC-δ and PKC-ε, which are involved in inhibiting the kinase activity of the receptor to insulin and thus inhibit insulin signaling [92]. The phosphorylation of IRS-1 at the residue of Serine-1101 by PKC-θ showed a blockade of the insulin-stimulated IRS-1 tyrosine phosphorylation [98]; this determines the signaling of insulin (**Figure 6B**). In addition to DAG, another fatty intermediate that is involved in insulin resistance is ceramides that can act as a second messenger and can modulate the activity

of kinases, phosphatases, and some transcription factors. These ceramides reduce the phosphorylation of IRS-1 through insulin and, in turn, are involved in the activation of protein phosphatase (PP)-A2, which can inhibit the phosphorylation of the Akt2 protein in muscle, which influences glucose consumption, since it inhibits the translocation of the GLUT4 glucose transporter to the plasma membrane [99].

Ceramides also activate inflammatory pathways such as c-Jun N-terminal kinases (JNK) and transcription factor NF-κB. The activity of JNK has been related to the pathogenesis of insulin resistance by the phosphorylation of IRS-1 in the Serine-307 residue; when this phosphorylation occurs, the activity in the signaling of the ISR-1 is diminished [100]. Phosphorylation of serine residues has been associated with blocking IRS-1 and inhibiting IR/IRS interaction, thus promoting the degradation of the IRS-1 protein, and thus inducing a lack of insulin response, which generates an insulin resistance.

On the other hand, obesity has been associated with low-grade inflammation, where insulin signaling in the adipocyte and hepatocyte is inhibited through several mechanisms, one of which is the inhibition of IRS-1 and the signaling cascade of insulin. Another mechanism is the inhibition of peroxisome proliferator-activated receptor (PPAR)-γ function; this is a nuclear factor that activates lipid synthesis through inducing enzymes and proteins involved in lipogenesis and fat storage, so that inhibition contributes to insulin resistance [101]. The increase of free fatty acids involve another mechanism where these acids activate some receptors involved in the innate immune response; among these are the TLRs, the medium chain fatty acids that can activate the TLR4 and this in turn initiates the intracellular signaling cascade that culminates in the activation of NF-κB, JNK, and suppressors of cytokine signaling (SOCS) pathways, involved in the activation of inflammatory pathways that induce the expression of inflammatory molecules including cytokines, chemokines, and effectors of the innate immune response; these molecules contribute to insulin resistance associated with obesity [61, 99].

In a stage of obesity, macrophages infiltrate adipose tissue and are characterized by an increase in the number of pro-inflammatory M1 macrophages, as well as T helper 1 (Th1), Th17, and $CD8^+$ T lymphocytes. The CD8+ cells play an important role in the differentiation, activation, and migration of macrophages, while $TCD4^+$ lymphocytes and regulatory T cells are diminished [102]. Macrophages that infiltrate adipose tissue stimulate lipolysis and gluconeogenesis is stimulated through IL-6, thus causing hepatic insulin resistance. The adverse effects of inflammatory cells and mediators on adipocytes is to accelerate the transfer of lipids found in adipose tissue to tissues such as skeletal muscle and liver, which lead to ectopic lipid deposits leading to resistance to the insulin in these tissues causing a metabolic dysfunction [103].

5.6. Type 2 diabetes mellitus and obesity

Currently, it is estimated that approximately 415 million people have *diabetes*, of which more than 90% have T2MD. However, 318 million people have a preclinical state of impaired glucose regulation [104, 105], but intensive pharmacotherapy modifies the patient's lifestyle, since it can reverse or delay the development of T2DM [106, 107]. In humans, obesity is the most common cause for developing insulin resistance, which is a key component in the etiology of T2DM [108, 109].

T2DM has been considered as a metabolic disease. However, investigations of recent years have revealed that the inflammatory responses triggered by the production and the release of adipokines from WAT [110], as well as oxidative stress, are the most important factors related to the development of insulin resistance and the pathogenesis of T2DM [111]. Several investigations [112–116] have reported that the levels of the pro-inflammatory cytokines increase abnormally in both experimental animal models and patients with insulin-resistant and T2DM compared with normal controls, which demonstrates the role of pro-inflammatory cytokines in the development of insulin resistance and the pathogenesis of T2DM [117, 118]. Among the various pro-inflammatory cytokines, TNF-α is one of the most important pro-inflammatory mediators that is critically involved in the development of insulin resistance and the pathogenesis of T2DM [111, 119]. The elevated level of TNF-α from tissue macrophages [120] induces insulin resistance in adipocytes and peripheral tissues by altering insulin signaling through serine phosphorylation that leads to the development of T2DM [119].

On the other hand, recently, epigenetic factors have been implicated in the regulation of genes that are involved in the development of obesity; these factors are: changes in DNA methylation and microRNAs (miRNAs) expression non-coding miRNAs. Although multiple risk factors have been related to the development of obesity, however, it has not yet been determined how these factors interact with each other in order to develop this disease [121].

miRNAs are a family of small non-coding endogenous RNA molecules approximately 17 to 25 nucleotides in length, which function as epigenetic regulators, modulating gene expression at the post-transcriptional level, without altering the DNA sequence. The miRNAs promotes the association of a protein complex, called the RNA-induced silencing complex (RISC), which directs the miRNAs to their target mRNA. The miRNAs generally bind to the 3′ untranslated region (UTR) of target mRNAs, through base pairing between a small fraction of the miRNA sequence, called the "seed" region composed of 2–7 nucleotides, to decrease gene expression by either of the two post-transcriptional mechanisms: translational repression or rapid degradation of mRNA [122, 123].

Apart from their endogenous actions, miRNAs can be secreted into the extracellular space inside nanoparticles called Exos [124, 125]. These Exos contain numerous miRNAs, which can function locally or can enter the circulation to act in different sites. In addition, these Exos can be incorporated into other cells to modulate their function [126, 127].

Finally, a recent study reported that adipose tissue macrophages (ATM) of obese animals secrete Exos that contains miRNAs, which can be absorbed in insulin target cells, both in vitro and in vivo models, resulting in cellular and systemic insulin resistance, as well as intolerance to glucose. In contrast, the treatment of obese recipients with ATM-Exos derived from lean mice leads to an improvement in glucose tolerance and insulin sensitivity, both in vivo and in vitro. This study demonstrated that miR-155 is among the differentially expressed miRNAs in obese ATM-Exos, and that this miR-155 can inhibit insulin signaling and glucose tolerance [128], probably through a mechanism related to the suppression of its target gene peroxisome proliferator-activated receptor (PPAR)-γ [129], since when PPAR-γ is stimulated, it causes insulin sensitivity; whereas if it is inhibited, it causes insulin resistance [130] (**Figure 7**).

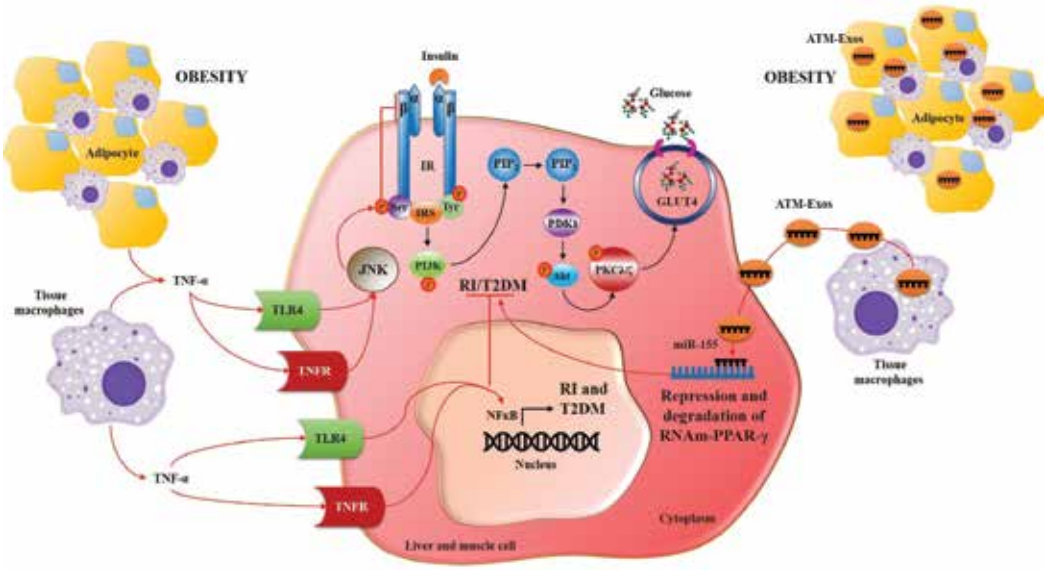

Figure 7. Relationship between type 2 *diabetes mellitus* and obesity. TNF-α from tissue macrophages induces insulin resistance by altering insulin signaling, which leads to development of T2DM. miR-155 inhibits insulin signaling and glucose tolerance, through of the suppression of PPAR-γ IR, insulin receptor; IRS, insulin receptor substrates; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol-3,4-diphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PDK-1, protein kinase 1; Akt, serine/threonine-specific protein kinase; GLUT4, glucose transporter type 4; FFA, free fatty acids; DAG, diacylglycerol; PKC, protein kinase C; JNK, C-Jun N-terminal kinases; TLR4, Toll-like receptor 4; T2DM, type 2 *diabetes mellitus*; TNFR, TNF receptor; IR, insulin resistance, PPAR-γ, peroxisome proliferator-activated receptor-γ; ATM, tissue macrophages. Description in the text.

6. Conclusion

It is very important to understand that adipose tissue is not only a fat storage and insulation organ, but it is an endocrine tissue capable of producing a great variety of molecules, which is associated with other cells and has a close and complex relationship with the immune system. The imbalance in homeostasis, hyperplasia, and overproduction of adipokines leads to pathological conditions, such as obesity and adipose tissue inflammation that can develop insulin resistance and favor the pathogenesis of T2DM.

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Conflict of interest

We have no conflict of interest related to this work.

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Chapter 7

Physical and Mental Health Consequences of Obesity in Women

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Additional information is available at the end of the chapter

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Abstract

Obesity and overweight are major health concerns and the leading preventable cause of death in developed and developing countries. Obesity affects men and women differently due to biological, socioeconomic, cultural and country-specific gender-related disparities. This book chapter outlines obesity as a risk factor for physical diseases and mental health disorders in women. Obesity has been shown to contribute to the risk of certain types of cancer, including breast, endometrial, gallbladder, oesophageal and renal cancer. In terms of reproductive health, obesity negatively affects both fertility and contraception. In addition, obesity is associated with early miscarriage, higher rates of caesarean section and high-risk obstetrical conditions, in addition to higher maternal and neonatal mortality rates, and congenital malformations. In terms of mental health, obesity is closely linked to depression, anxiety disorders, neurodegenerative diseases and sleep disorders. Socioeconomic, psychosocial and behavioural factors, factors associated with ageing, mechanisms related to the microbiome, gastrointestinal and vascular system, intracellular pathophysiology and metabolism in the body, hormones, adipocytokines and problems associated with medical treatment are important factors linking obesity with its negative consequences on physical and mental health.

Keywords: obesity, women, women's health, gender differences, sex hormones, menopausal status, cancer, metabolic syndrome, diabetes, vascular disease, osteoarthritis, reproductive health, fertility, pregnancy, psychiatric diseases, depression, posttraumatic stress disorder, dementia, insomnia

1. Introduction

1.1. Obesity is related to major health issues in women

In 2016, 13% of the world's population (15% of women and 11% of men) was affected by obesity, which is characterised by excess body fat with a Body Mass Index (BMI) ≥ 30 kg/m^2. Obesity and overweight (BMI ≥ 25 kg/m^2) are a major health concern, and the leading preventable cause of death in developed and developing countries. Obesity leads to severe impairment of health in both genders [1]. However, obesity may pose a gender-specific risk to the development of comorbidities [2], and can be considered as both an influencing and resulting factor of at least seven of the top 10 health issues in women published by the World Health Organisation (WHO) in 2015: Cancer, reproductive health, maternal health, human immunodeficiency virus infection, other sexually transmitted infections, violence against women, mental health, non-communicable diseases, being young and getting older [3], because obesity is associated with breast and cervical cancer, sexual, reproductive and maternal health issues, developmental and ageing difficulties, mental health disorders, and non-communicable diseases, including obesity itself and its metabolic consequences.

Obesity has been consistently shown to increase rates of breast cancer in postmenopausal women, and is associated with poorer survival rates and increased likelihood of recurrence [4–6]. In addition, a systematic review reported a positive correlation between BMI and endometrial, gallbladder, oesophageal adenocarcinoma and renal cancer in women [7]. In terms of reproductive health, obesity negatively affects both fertility and contraception due to hormonal and metabolic alterations, including hyperinsulinemia and hyperleptinemia, insulin resistance (IR) and hyperandrogenism [8, 9]. One of the most common reproductive disorders in women of childbearing age, and the leading cause of infertility, is polycystic ovarian syndrome (PCOS), which can be impaired or even caused by visceral obesity [10]. Maternal health has been reported to worsen as a result of obesity or abnormal weight gain during pregnancy [11–13]. Obesity is related to early loss of pregnancy, higher rates of caesarean (c-) section and high-risk obstetrical conditions, in addition to higher maternal and neonatal mortality rates and congenital malformations [8, 14–16]. As one of the most common mental health problems in women, according to the WHO, depression is closely linked to obesity in a vicious cycle [17, 18]. In general, poor socioeconomic status, low income and experience of violence are gender-specific risk factors for common mental health disorders in women, including depression, anxiety, posttraumatic stress disorder (PTSD) and dementia, which also promote obesity [18–20]. Low-grade systemic inflammation deriving from adipose tissue (AT) also appears to be a major factor contributing to the pathophysiology of type II diabetes mellitus (DM II), metabolic syndrome (MS) and cardiovascular diseases (CVD), in addition to the above-mentioned psychiatric disorders [21–24].

1.2. Specific aspects of obesity in women

Obesity affects men and women differently due to biological, socioeconomic, cultural and gender-related disparities. Sex hormones have a marked impact on metabolism by modulating the production and effects of hormones and cytokines in the AT, which are important messenger molecules of the immune system [25, 26]. Sex hormones steer patterns of fat distribution. Men

usually exhibit visceral fat accumulation, which is considered to be metabolically unhealthy, whereas fat accumulates subcutaneously in women. However, the fat distribution in women is influenced by menopausal status, with several studies consistently showing that the prevalence of MS in women increases alongside post-menopausal changes in sex hormone production [27].

In addition to the above, women appear to be more likely than men to develop MS as a response to work stress and low socioeconomic status [2]. In developing countries with more often conservative societies, more women have a sedentary lifestyle than men, and performing physical activity (PA) in public areas is more restricted in women. Despite westernisation, obesity is often a more culturally accepted body image in these countries. Additionally, women may have different food preferences to men, as they tend to consume more foods high in added sugars, refined carbohydrates and energy density. At present, there is a higher availability of these types of food due to the economic growth of developing areas leading to an influx of processed food products [28].

1.3. Aim of this review

This review summarises the physical and mental health consequences of obesity in women. It also compiles the mechanisms by which obesity may lead to these problems.

Due to space limitations, this book chapter does not assess the quality of the cited studies, nor does it intend to cover all the consequences of obesity. Therefore, the sequelae of obesity, which are equally present in men and women, are not a focus of this chapter.

2. Physical health consequences of obesity in women

2.1. Cancer

Obesity has been found to increase cancer risk and affect survival rates in both genders [29–31]. However, the increased risk of different types of cancer varies among men and women. The WHO states that breast, cervical, colorectal, lung and stomach cancer are leading in women's cancer statistics [32]. A systematic review and meta-analysis of prospective observational studies showed that an increase of BMI by 5 kg/m^2 was strongly associated with an elevated risk of endometrial, gallbladder, oesophageal, and kidney cancer in women. A weaker positive association was found between BMI and postmenopausal breast, pancreatic, thyroid and colon cancer [7]. In addition, obesity was found to increase mortality rates for breast, uterine, cervical and ovarian cancer in women [33]. The higher mortality rates may be a result of delayed screenings, obesity-associated comorbidities, poorer treatment effects, and increased surgical and radiotherapy complications in obese women [8].

2.1.1. Mechanisms linking obesity and cancer

Various mechanisms are suggested to link obesity with cancer. In the ATs of severely obese individuals, the inability of adipocytes to further expand may lead to inflammatory triggers, including hypoxia following cell death, free fatty acid release and a systematic inflammatory

status [34, 35]. These inflammatory conditions may have tumour-promoting effects by inducing inflammatory cascades via the production of cytokines [36–38]. Additionally, local inflammatory processes in AT can directly contribute to cancer development. For example, a previous study examined signs of mammary tissue inflammation, including increased activity of macrophages in patients with early-stage breast cancer [5]. Inflammation and tumour growth may also be promoted via imbalance in the gut microbiome, the composition of which can be negatively influenced by a high-fat diet (HFD). Dysbiosis within the microbiome has been suggested to lead to elevated gut permeability [39, 40]. In this process, bacterial components appear to activate immune receptors, including Toll-like receptor 4, which contributes to the systemic inflammatory response in obesity [38].

Under premenopausal conditions, obese women may be better protected against inflammation originating from AT as they have a more favourable subcutaneous fat, rather than visceral fat storage [41, 42]. Visceral AT has repeatedly been demonstrated to be more systematically harmful [35, 43]. In addition, the female sex hormone, oestrogen, is considered to support an anti-inflammatory immune response [26] and to decrease the production of pro-inflammatory cytokines, including interleukin (IL)-6 and tumour necrosis factor-α (TNF-α), and thereby protect against carcinogenesis [44, 45]. However, there is an alteration in hormone status in women during menopause, with rapid hypoestrogenemia, relative hyperandrogenemia and a decrease in the hepatic production of sex-hormone binding globulin (SHBG), which increases the bioavailability of androgens [46]. This is accompanied by the accumulation of visceral fat independent of the increase in subcutaneous fat due to increasing age [47, 48]. Therefore, women do not appear to be protected by the anti-inflammatory effects of oestrogen, which renders them more susceptible to post-menopausal metabolic complications and cancer [37, 49].

If the oestrogen levels of obese and non-obese postmenopausal women are compared, the oestrogen levels in obese postmenopausal women are higher than in their non-obese peers. This is explained by aromatase activity in AT, which catalyses the conversion of testosterone into estrogens [50, 51]. In addition, aromatase activity is exponentiated by inflammatory mediators derived from AT, including prostaglandin E2, IL-6 and TNF-α [52, 53]. Despite the anti-inflammatory features of oestrogen, these relatively high oestrogen levels in obese postmenopausal women have been suggested to increase the risk of oestrogen-dependent breast cancer after menopause [6, 51, 52, 54].

The gut microbiome also appears to be capable of influencing oestrogen levels, possibly by enzymatic deconjugation, and thus contributing to cancer development [40]. However, the underlying mechanisms remain to be fully elucidated.

The metabolic comorbidities of obesity itself also increase cancer rates. An increased cancer rate in connection with DM II has been observed in liver, endometrial, pancreatic, colorectal, bladder, and breast cancer [55].

2.1.2. Breast cancer

Breast cancer is the leading type of cancer among women aged 20–59 years worldwide [56]. The obesity-related risk of developing breast cancer varies depending on menopausal and hormone

receptor status [6, 40]. The majority of studies have shown that obese women are at increased risk of oestrogen receptor (ER)-positive postmenopausal cancer [57]. ER-positive breast cancer is the most common subtype and accounts for 70% of all breast cancer cases [40, 58].

Previous studies have found central adiposity to be an independent predictor of both postmenopausal and premenopausal breast cancer risk [59–61]. Obesity has been consistently shown to increase rates of breast cancer in ER-positive postmenopausal women by 30 to 50%. Weight gain in young adults has also been found to be associated with an elevated risk of postmenopausal ER-positive breast cancer, whereas weight loss, bariatric surgery and PA are consistently associated with reduced risk [6, 62–64]. Correspondingly, biological markers associated with breast cancer, including oestrogen, SHBG, CRP and IL-6, have been found to decrease following weight loss and as a consequence of PA [65, 66]. Conversely, the tumour growth rates of mice fed an HFD have been reported to increase compared with those in normally fed mice when inoculated with breast cancer cells [67].

Studies of breast cancer mortality and survival rates have noted that adiposity is associated with reduced survival rates and higher rates of recurrence, irrespective of menopausal status and after adjustment for stage and treatment [68–72]. Additionally, obese patients with breast cancer often receive suboptimal treatment in terms of the doses of chemotherapy [73] and treatment appears to be less effective in patients with a BMI > 30 kg/m^2 [74].

The use of menopausal and postmenopausal hormone therapy (MHT) to mitigate unwanted menopause-related symptoms has been reported to increase ER-positive breast cancer risk; these estrogens may accumulate in mammary AT [75].

2.1.3. Cervical cancer

Cervical cancer is the second leading cancer type in women and is linked to the sexually transmitted infection, human papillomavirus [56]. Studies investigating the connection between obesity and cervical cancer have been limited and inconsistent [29, 33]. A published meta-analysis of nine studies with 128, 233 participants found no association between overweight, but a weak association between obese individuals and the risk of cervical cancer [76]. However, investigations have repeatedly shown that obese women are more likely to miss screening examinations, possibly due to embarrassment and discomfort [77, 78]. This may partly explain the higher mortality rates of obese patients with cervical cancer [33]. According to the subtypes of cervical cancer, obesity appears to be involved in the development of cervical adenocarcinoma rather than squamous cell carcinoma [8, 79].

2.1.4. Endometrial cancer

Adiposity has been established as a factor closely related to endometrial cancer risk and mortality rates [7, 31, 80, 81]. A prospective study recruiting 1.2 million women in the United Kingdom found that ~50% of the cases of endometrial cancer were attributable to being overweight or obese, and it was reported that the risk of endometrial cancer increased linearly with increasing BMI [30]. It has been estimated that there is a 2- to 4-fold increased risk of endometrial cancer in overweight or obese women [8, 29, 82]. As a causal mechanism for the

association between obesity and endometrial cancer, increased circulating oestrogen levels, insulin resistance and inflammatory processes in AT have been proposed [82, 83]. In particular, unopposed oestrogen-its application in the absence of progesterone-has been shown to lead to an increased cancer risk via inducing the mitotic activity of endometrial cells [84]. Therefore, in premenopausal cancer, progesterone deficiency, rather than an excess of oestrogen, may be responsible for the effect of obesity on cancer risk [30]. Hyperleptinemia occurring in obesity has been reported to be involved in ovarian steroidogenesis, and leptin treatment in mice decreases progesterone levels [85]. Additionally, obesity-associated PCOS (see Section 2.3.1.) with anovulation or oligoovulation has been discussed as a possible consequence of chronic exposure to unopposed oestrogen [8].

2.1.5. Kidney cancer

The risk of renal cell cancer in overweight and obese men and women, compared with those of a normal weight, appears to be 1.5- to 2.5-fold higher in study populations [29]. Kidney cancer has been reported to show a dose-response relationship with increasing BMI [86]. The increase in risk with increasing BMI appears to be higher in women than in men and independent of blood pressure [87–89]. The positive association with obesity is predominantly found in clear cell type kidney cancer, which is the dominant histological subtype [89]. In contrast to breast and endometrial cancer, the risk of developing kidney cancer appears to be decreased in the presence of oestrogen, as ER activity inhibits renal cell carcinoma growth [90]. Therefore, metabolic-, inflammatory- and adipokine-related features of obesity may mediate the higher risk of kidney cancer. Kidney cancer is often found in diabetic individuals with hyperinsulinemia and hyperglycaemia, which are considered to be carcinogenic factors by generating increasing levels of pro-inflammatory cytokines, reactive oxygen species and lipid peroxidation [91]. AT-derived hormones, including leptin and insulin-like growth factor-1, contribute to the direct effect of obesity on kidney cancer [92], and elevated leptin levels have been be demonstrated to cause renal fibrosis directly [93]. Obesity is also likely to lead to glomerulopathy by increasing blood flow, arterial pressure, activation of the renin-angiotensin-aldosterone system and consequently glomerular hyperfiltration, which, in turn, leads to microalbuminuria and loss of renal function [94, 95].

2.1.6. Gastrointestinal cancer

As gastrointestinal cancer is among the leading cancer types in women, it is worth mentioning that obesity increases their risk of incidence according to several studies. Obesity appears to be associated with a 2- to 3-fold increase in risk for adenocarcinoma of the oesophagus [29]. A higher BMI often leads to gastro-oesophageal reflux, and it is hypothesised that an increased occurrence of reflux explains the association between obesity and oesophagal adenocarcinoma [96]. Reflux is provoked by visceral fat accumulation with increasing pressure inside the abdominal cavity [97]. Again, women are more prone to this type of cancer after the menopause due to their redistribution of fat owing to the hypoestrogenic condition [98]. Similarly, an approximately 2-fold elevated risk of gallbladder cancer in women, but not in men, has been consistently demonstrated in previous studies [29]. It has been suggested that obesity provokes the development of gallstones, inducing local inflammatory stimuli. Gallstones and gallbladder cancer

share common risk factors, including female gender [99]. Obesity increases the risk of stomach cancer, a common type of cancer in women [29, 56]. This appears to be especially true for gastric cardia rather than non-cardia gastric cancer [29, 100, 101], however, no gender differences have been found [101]. Colorectal Cancer (CRC) is the second most common type of cancer contributing to mortality rates in both men and women. Although obesity has been shown to be a more significant risk factor for colon cancer in men rather than women, there is a striking percentage of 18% by which an increase of 5 kg/m^2 in BMI can elevate the risk for colon cancer in both genders [102, 103]. The linear association between BMI and CRC is stronger in premenopausal than postmenopausal women [102]. No differences in overall survival, survival by stage, or local or distant recurrence of CRC have been found between genders [104].

2.2. Metabolic disorders

2.2.1. Metabolic syndrome (MS) and menopausal status

According to the International Diabetes Federation, MS is characterised by a cluster of metabolic abnormalities, and increased blood pressure MS is associated with an increased risk of DM II, CVD and cerebrovascular disease (CeVD) [105]. Obesity and MS are significantly more prevalent in postmenopausal women, compared with men of the same age, and the increase in MS prevalence with age is more marked in women than in men [2, 40, 106]. In premenopausal women, PCOS appears to be a frequent clinical abnormality associated with MS [107].

Both endogenous and exogenous factors contribute to the association between weight gain during menopause and the occurrence of MS, including changing hormonal status accompanied by fat redistribution, physical inactivity, lower energy expenditure, unhealthy nutrition, medications (psychotropic drugs, insulin and steroids) and diseases [46, 108]. Unlike men, women have also been shown to develop MS as a response to work stress [2].

Possibly the most crucial link between MS and menopausal status is the essential role of oestrogen in the regulation of metabolic homeostasis. Under the influence of hormonal oestrogen, women are metabolically healthier than men; they exhibit higher insulin sensitivity, higher levels of high-density lipoprotein (HDL), and lower levels of triglyceride (TG) and low-density lipoprotein (LDL)-cholesterol, as well as beneficial subcutaneous fat distribution and inhibited lipogenesis by suppressed lipoprotein-lipase activity [108–111]. In contrast, rodent experiments show that, following menopause or ovariectomy, there is a marked decline in insulin sensitivity alongside an increase in fat mass, and elevations in circulating inflammatory markers, LDL, TG and fatty acids [108]. Unfortunately, aromatase-derived estrogens in obesity do not protect women from metabolic disturbances [46]. This is possibly due to the finding that ER-expression changes during menopause and alterations of ER in AT affect inflammatory processes and the distribution of fat regardless of circulating estrogens [112]. It has been suggested that only oestrogen levels in stable physiological concentrations are metabolically favourable, whereas supraphysiological levels or the overstimulation of ER may induce IR and DM II [108]. The relative surplus of androgens-from continued production in the adrenal gland-in conditions lacking estrogens may also contribute to the onset of MS, particularly regarding IR and the growth of visceral fat [113]. Gonadotropins, which are elevated following menopause in response to the peripheral drop of hormones, stimulate further androgen

synthesis. Low levels of SHBG also appear to have a significant impact on the progress of IR, whereas hyperinsulinemia itself can boost ovarian androgen production [46].

There is evidence from animal experiments that, with the exception of sex hormones and gonads, different sex chromosome makeup may also contribute to differences in food intake, fat accumulation, fatty liver, hyperinsulinemia and hyperlipidemia between men and women [42]. However, in studies involving humans, physiological changes during the menopause appear to represent the most crucial gender-related factor for the increased prevalence of MS in women following the menopause in comparison to their age-matched male counterparts.

2.2.2. Type II diabetes (DM II)

Being overweight or obese is considered to be the main risk factor for developing DM II. A previous study found that, if a woman has a BMI of ≥25 kg/m^2, her relative risk of DM II is ~5, whereas the risk for a man is between 2 and 3 [114]. With MS as a predictor, it is not surprising that the prevalence of DM II in women after the menopause increases at a higher rate than that of men [115]. However, other conditions and diseases are more prevalent at this time in a women's life, including sleep disturbances and depression, which are independent risk factors for diabetes [113]. Substantial evidence highlights that the risk of the DM II depends on the onset of menopause; a natural menopause with an average onset age of ~50 years does not appear to affect the occurrence of DM II, whereas premature menopause, regardless of whether it is caused naturally or surgically by hysterectomy with bilateral oophorectomy, increases the risk of DM II [106]. In contrast, premenopausal women have a reduced incidence of DM II compared with age-matched men [108].

Large observational studies and a long-term randomised controlled trial examining the use of MHT in women with diabetes found an improved disease outcome, corroborating the oestrogen-deficiency theory in middle-aged women [106, 116]. However, study results are conflicting [117, 118] and current knowledge is not sufficient to recommend the use of MHT in women with MS or diabetes, with CVD risk evaluation being advised prior to initiating MHT [106].

2.3. Cardiovascular and cerebrovascular diseases

Although evidence suggests that women have superior protection against CVD and experience CVD events on average 8 years later than men, CVD remains primary cause of death in women, accounting for 46% of deaths in older women worldwide [2, 56]. Protection against CVD is reversed in the presence of DM II, when the risk of CVD is almost doubled in men and more than three times higher in women [2]. Notably, the development of cardiac steatosis, a risk factor for heart failure, is more pronounced in the presence of impaired glucose tolerance (IGT) [119, 120]. In CeVD in women, a prospective population-based study investigating 8419 participants aged >55 years showed that the cumulative incidence of CeVD was higher in women than in men [121]. Prospective studies and meta-analyses have found that both hyperglycaemia and hyperinsulinemia increase the risk of stroke, particularly in women [2, 122]. Additionally, the diagnosing of CeVD events appears to be delayed in women due to the less traditional stroke symptoms, including impaired consciousness and altered mental status [123].

In terms of cerebrovascular and cardiovascular health, oestrogen is described to enhance endothelial function and vasodilatory effects by increasing prostacyclin and nitric oxide levels, protecting the endothelium from TNF-α-induced inflammation and downregulating levels of plasma LDL-cholesterol, and is considered to be neuroprotective [40, 46, 122, 124]. Accordingly, several studies have found that low-dose transdermal oestrogen application early following menopause was associated with attenuated risk of stroke [122].

2.4. Osteoarthritis

Osteoarthritis (OA) is a disease characterised by joint pain, stiffness and impaired movement. Menopausal women have an increased risk for OA. One study calculated the incidence rates of OA in men and women according to age for different joints and found incidence rates for knee, hip and hand arthritis higher in women [125, 126]. Prevalence rates of arthritis are related to body weight. A BMI increase by 5 kg/m^2 is associated with a 36% higher risk of OA. Systemic inflammation originating from the AT seems to be a crucial factor for the development of OA [127, 128].

2.5. Reproductive health

Obesity influences the onset of puberty as gonadal function is regulated by metabolic status. Insulin and leptin indirectly impinge hypothalamic neurons emitting gonadotropin-releasing hormone, a hierarchical hormone within the hypothalamic-pituitary-gonadal (HPG) axis [129, 130]. As the prevalence of childhood obesity has increased, a study found that the median age at menarche decreased by 3–5.5 months in the US between the late 1960s and 1990 [8].

In patients with PCOS, the age at menarche is ascertained to be ~6 months earlier than in unaffected girls, and premature pubarche is suggested to be the earliest manifestation of PCOS. As with premature pubarche, PCOS is often found in obese individuals, with only ~20% of cases of PCOS in non-obese individuals [8]. Both obesity and PCOS are associated with irregularities in the menstrual cycle and disturbed metabolic features, including IR, increased leptin, and decreased adiponectin levels [8, 131]. PCOS is clinically defined by the features hyperandrogenism, oligo- or anovulatory cycles and polycystic ovary [132]. Disturbed insulin sensitivity and hyperinsulinemia are widely believed to be the underlying causes of PCOS [133]. In particular, abdominal obesity combined with hyperinsulinemia is known to cause decreased SHBG levels and stimulate androgen production within the ovary, and possibly within the zona reticularis in the adrenal cortex [134–137]. Hyperleptinemia was found to be positively correlated with androgen levels in women with PCOS, and hyperleptinemic mice presented with prolonged menstrual cycles, atrophic ovary and reduced hypothalamic gonadotropin-releasing hormone at an older age [138, 139]. Leptin exerts its hypothalamic neuroregulatory function indirectly via interneuronal pathways, which can be attenuated by the leptin resistance typically found in response to hyperleptinemia in obese subjects [139–141]. Additionally, chronic elevation of circulating estrogens due to aromatisation in AT disturbs the HPG axis [142]. Taken together, obesity disrupts the ovulatory process via metabolic abnormalities affecting both peripheral and central hormonal derangements.

A Nurses Health Study, which included 116,000 women, investigated ovulatory failure and menstrual cycle irregularity in women with and without PCOS, and reported an increased relative risk of infertility with increased BMI in all women [143]. Another study of 22,840 women reported reduced fecundity, even in obese women with regular cycles [8]. In obese women without PCOS, assisted reproduction is less successful and higher doses of gonadotropins are required for ovarian stimulation compared with woman of a normal weight [144].

Apart from the above-mentioned biological factors, social factors are also suggested to affect the reproductive condition of women. For example, a long-term study indicated that obese American women have lower fertility, partly due to a lower probability of marriage [145].

Contraception also appears to be negatively affected by obesity; studies have found a higher risk of contraception failure in heavier subjects when investigating different methods of contraception, including oral, transdermal and local hormonal contraception [8].

2.6. Maternal health

Being young was stated by the WHO to be one of the 10 highest health risks for women. This can partly be explained by the high number of deaths due to complications during pregnancy and childbirth, particularly in developing countries [3]. Obesity compromises maternal health by increasing the number of pregnancy-related ante-, peri- and postpartum complications. In addition, obesity holds potential for intergenerational programming, meaning that maternal obesity can increase the likelihood of weight gain with metabolic consequences and CVD risk in the adult life of the offspring [8, 143, 146]. In Europe and the US, 20–40% of pregnant women are considered to be obese, due to the high prevalence of obesity, or they gain excessive weight during pregnancy [147]. In 2008, the prevalence of overweight and obesity in developing areas, including Africa, reached 40% in women of childbearing age [148].

Preconceptional obesity elevates the risk of gestational diabetes (GDM), gestational hypertension (GH), preeclampsia (PE) and deep venous thromboembolism (DVT), all of which are related to higher maternal morbidity rates postpartum [8, 14, 143, 149]. A previous study assessed metabolic complications in relation to prepregnancy overweight and obesity (BMI ≥ 30 kg/m^2) 10 years postpartum in premenopausal Chilean women. At 10 years post-delivery, women who were overweight and obese prior to pregnancy had significantly higher rates of IR, abdominal obesity and hypertriglyceridemia, compared with women of normal weight prepregnancy [150]. The risk of suffering from future DM II is higher for women with GDM than for those without. A meta-analysis revealed a 7-fold increased risk of DM II in women with GDM, compared with women without GDM [151]. A systematic literature review examined the incidence of DM II in women suffering from GDM and reported that the cumulative incidence increased steeply within 5 years of delivery and levelled off after 10 years [152]. Obese women were found to be 4.5–8.7 times more likely to develop GH [153]. GH increases both maternal and foetal mortality with a 5- and 3-fold higher risk for PE and stillbirth, respectively [145]. PE, typically a late pregnancy or post-delivery syndrome characterised by new onset of hypertension and proteinuria, can often appear superimposed on established gestational or prior hypertension, and is frequently observed on a background of pre-existing maternal morbidities, including obesity [149]. Obese pregnant women are estimated to be at 3–10 times higher risk of PE [14]. PE increases the mortality rates of women

during or shortly after pregnancy through severe complications, including eclampsia or HELLP syndrome, which is a life-threatening complication of pregnancy with haemolysis, elevated liver enzyme levels, and low platelet count. Excessive hypertension can also affect cerebral autoregulation and lead to permanent damage or death via cerebral haemorrhage [149]. During and after pregnancy, obesity increases the risk of DVT 4- to 5-fold [14].

Obesity is related to higher rates of miscarriage and preterm birth (PTB). A systematic review of the literature examining the association between BMI and PTB found an increase in the risk of PTB at different gestational ages in obese women, which was even higher for early PTB (<32 weeks) in morbidly obese women (BMI >40) [154]. Additionally, obesity in women is associated with post-date delivery and a higher mean birth weight [155].

At the time of birth, obese women more often require assistance in delivery, for example induction of labour or c-section [8, 14]. Failure to progress with labour, administration of oxytocin, and epidural anaesthesia were more frequently experienced by obese women [155]. Emergency c-sections are more common among obese mothers due to macrosomia of the foetus which, in turn, results from IGT of the obese mother [143, 156]. The odds ratio (OR) of c-section was increased from 1.43 to 2.36 when comparing morbidly obese and non-obese females [155]. Rates of postpartum haemorrhage, infection and venous thromboembolism are also elevated in obese women [14, 15, 156].

2.7. Neonatal health

Newborns are also affected by morbid maternal obesity, with higher neonatal mortality rates owing to higher risk of neonatal complications, including hypoglycaemia, hyperbilirubinemia, birth injury, infections and respiratory distress syndrome [157, 158]. They are also more likely to suffer from malformations, including neural tube defects, spina bifida, cardiovascular anomalies, and cleft lip and palate. Intriguingly, the prevalence of gastroschisis in neonates was documented to be lower among obese mothers [8].

Even if obese mothers experience an uneventful pregnancy and delivery, the postpartum effects of obesity on the infant's and mother's lives often continue. Difficulties with breastfeeding are commonly observed between newborns and their obese mothers [14]. Among obese mothers, a lower prolactin response to suckling was observed leading to a delay of milk production and, thus, decreasing rates of breastfeeding initiation [159]. A recent investigation assessed intention and initiation of breastfeeding in different prepregnancy obesity classes according to BMI (normal: 18–24.9 kg/m^2, overweight: 25–29.9 kg/m^2, obese: 30–34.9 kg/m^2, very obese: 35–39.9 kg/m^2, and extremely obese: ≥ 40 kg/m^2). The authors noted that both intention and initiation were significantly lower among women with extreme obesity, while figures were similar and reduced only slightly reduced from normal to very obese women. In terms of intention to breastfeed, declared antepartum by study participants, extremely obese women were often younger, less well-educated, smokers and African American, compared with women in the other obesity classes suggesting that social and ethnic factors may play an additional role [160]. Similar figures and the inverse correlation between initiation of breastfeeding and higher classes of obesity were also confirmed in another study involving 8430 breastfeeding women [161]. In addition, the majority of studies observed a shortened duration and discontinuation of breastfeeding

Aspects of physical health affected by obesity	Specific consequences
Cancer	ER-positive postmenopausal breast cancer
	Endometrial cancer
	Kidney cancer
	Oesophageal adenocarcinoma
	Gallbladder cancer
	Gastric cardia cancer
	Colorectal cancer
Metabolic disorders	Metabolic syndrome
	DM II
Vascular health	CVD
	CeVD
Reproductive health	Premature pubarche
	PCOS
	Infertility
Skeletal system	OA
Maternal health	GDM
	GH, PE, eclampsia
	HELLP syndrome
	DVT
	Foetal macrosomia
	Miscarriage, stillbirth
	Maternal mortality ↑
	PTB, post-date delivery
	Postpartum haemorrhage
	Postpartum infection
	Breastfeeding difficulties
	More frequent need for:
	• Induction of labour
	• c-section
Neonatal health	Frequent health consequences in newborns of obese mothers:
	• Increased neonatal mortality
	• Malformations
	• Birth injury
	• Hypoglycaemia
	• Hyperbilirubinemia
	• Respiratory distress syndrome

For details regarding the association between these physical health issues and obesity see Section 2 "Mental health consequences associated with obesity in women". Abbreviations: oestrogen receptor (ER), diabetes mellitus type II (DM II), cardiovascular disease (CVD), cerebrovascular disease (CeVD), polycystic ovary syndrome (PCOS), osteoarthritis (OA), gestational diabetes (GDM), gestational hypertension (GH), preeclampsia (PE), haemolysis, elevated liver enzymes and low platelet count (HELLP), deep venous thromboembolism (DVT), increase (↑), preterm birth (PTB), caesarean (c-) section.

Table 1. Physical health consequences of obesity in women.

among obese women, even on adjustment for confounding factors [162]. Investigations assessing the maternal health consequences of breastfeeding found lower levels of fasting plasma glucose and insulin, as well as lower rates of diabetes and IGT in breastfeeding women [151].

As already mentioned, an unfavourably metabolic, intrauterine environment, for example promoted by an obese mother, can elevate the risk of developing obesity and related disorders in adulthood. For example, studies have shown that children of diabetic mothers had an almost 10 times greater risk of IGT at the age of 10–16 years, compared with controls, and the prevalence of obesity in children of diabetic mothers was higher than that in children whose mothers were non-diabetic, irrespective of maternal BMI. This intergenerational transition may contribute to the global epidemic of obesity [143]. **Table 1** summarises the physical health consequences of obesity in women discussed in this section.

3. Mental health consequences of obesity in women

3.1. Depression

The gender difference in depression appears consistently in psychiatric epidemiology. A comprehensive review of general population studies revealed that major depression rates are more predominant in women than in men. Furthermore, depression seems to be more persistent in women, and female gender is a significant predictor of recurrence [18]. For depression, the female-to-male ratio was previously described as 1.5 [163]. Data from the general practice research database, which contains linked anonymised records of over 3 million patients registered in the UK, show that the incidence of depression in women is about twice as high as it is in men [164].

Irrespective of gender, obesity is regarded as an independent risk factor for depression and vice versa. A meta-analysis of studies examining the association between obesity and depression found an OR of ~1.3–1.4 for depression in obesity, and an OR of 1.7 for the inverse relationship [165]. Broadly, the association becomes more marked with increasing severity of obesity [8, 165–167]. However, continued dieting is also seen as a risk factor for depression [166].

Different biological and psychosocial mechanisms are suggested to link obesity and depression, some of which are considered to be more distinctive in women. An upregulation of inflammatory mediators, including TNF-α, has been shown repeatedly in obesity and depression, and may be a causal link between the two diseases. TNF-α has been found to activate indoleamine-2,3-dioxygenase, which degrades the serotonin precursor tryptophan leading to a central deficiency of serotonin [23, 24, 168]. Moreover, in both diseases, hormonal disturbances in the hypothalamic-pituitary-adrenal (HPA) axis are observed. Other hormones are also involved in the regulation of mood, appetite and the HPA axis, including leptin and ghrelin [166].

Particularly among women, who are considered to have a higher societal pressure to remain thin, obesity leads to poor self-image and self-esteem, as well as discrimination, which can promote

the development of depression [8, 165, 166, 169]. Conversely, depression brings about poor food choices, overeating and reduced exercise due to a lack of motivation, which, in turn, contributes to the progression of obesity [17, 170, 167].

It is often noted that a lack of PA is linked to depressive symptoms. Similarly, obese individuals show decreased PA, possibly due to limited function of their musculoskeletal system or sleep disturbances; this includes obstructive sleep apnoea syndrome (OSAS), which is accompanied by increased daytime sleepiness and elevated, pro-somnogenic cytokine levels [166, 171, 172]. However, a lack of physical activity may also be a causal factor contributing to the development of obesity.

As mentioned above, women across different age groups, ethnic backgrounds [20, 173–175], and specifically obese women [115, 176] are reported to be less physically active compared with their comparable male counterparts [28], which may lead to the development of depressive symptoms in addition to the burden of obesity [177].

Obesity in women may also be a consequence of psychiatric disorders. Women are 48% more likely than men to use any psychotropic medication following statistically controlling for demographics, health status, economic status and diagnosis [178]. In the US, data from the National Health and Nutrition Examination Surveys between 2005 and 2008 indicate that 11% of Americans aged ≥12 years take antidepressant medication, with taking 2.5 times as many antidepressants as males. Women received more antidepressant medication across all age groups, which is not an expression of the severity of depression; when comparing the same degrees of depression between women and men, women were more likely to receive a prescription for antidepressants [179]. For psychopharmacological agents, including tri- and tetracyclic antidepressants and mood stabilisers, weight gain is known to be a frequent side effect; certain drugs are associated with weight gain of up to 20 kg [180].

Within the lives of women, they appear to be particularly prone to depression during pregnancy and menopausal transition [113, 181]. At this life stage, the occurrence of obesity is simultaneously more likely. One review and meta-analysis involving 540,373 antenatal or postnatal women from countries worldwide, showed an increased OR of both antenatal (OR 1.43) and post-natal (OR 1.30) depression in obese women, compared with normal-weight women [153]. At the time of menopause, women often experience short-term changes in health and quality of life, involving sleep disturbance and affective symptoms, which are factors frequently causing women to seek medical attention [182]. Therefore, it is conceivable that women are prescribed more psychotropic drugs at this time, which may foster the progression of obesity in addition to the other factors mentioned above.

It has also been reported that oestrogen depletion can provoke depressive symptoms [40, 113].

3.2. Anxiety disorders

The male-to-female ratio for anxiety disorders is reported to be even higher than those for depression, ranging from 1.4 for any anxiety disorder to 1.7 for generalised anxiety, and up to 2.0 for panic disorder [163]. In terms of obesity, a recently published review reported inconsistent

findings on the relationship between obesity and anxiety disorders. Although some studies have demonstrated poor correlation, others nave found clinically significantly associations of panic disorder, specific phobias and social anxiety with obesity in females [165, 183]. The severity of obesity may influence these associations [183, 184].

In terms of the specific stages in women of pregnancy and menopause, another meta-analysis showed a higher risk for antenatal anxiety in obese women, compared with normal-weight women (OR 1.41), although there are few postnatal studies. The higher risk for antenatal anxiety has been explained by findings of qualitative research suggesting that women know of the elevated health risks associated with their obesity, which may increase their anxiety levels [153]. Only women with low, but not high, premenopausal anxiety levels have been shown to be more prone to developing anxiety during and after menopausal transition [185]. In relation to obesity, symptoms of anxiety were found to be associated with obesity neither pre- nor postmenopausally [184].

3.3. Posttraumatic stress disorder

The WHO estimated that 80% of the 50 million individuals experiencing trauma-related events, including violent conflicts and civil wars, are women and children, and the lifetime prevalence rates of violence against women vary between 16 and 50%. At least one in five women is affected by rape or attempted rape in their lifetime. These figures of trauma prevalence among women are associated with correspondingly high rates of PTSD in women [18]. For PTSD, the female-to-male ratio was 2.7, which was, alongside migraine, the highest ratio of all stress-related psychiatric disorders in a previous study [163]. Data from the Adult Psychiatric Morbidity Survey in England in 2014 showed the highest prevalence of PTSD in women aged 16–24 years, which was more than three times greater than in men [186]. There is evidence across several studies for an association between PTSD and obesity. A systematic review and meta-analysis of the available studies recorded an increased OR for obesity if individuals suffered from PTSD [187]. More severe symptoms, as well as early onset of PTSD, were found to be linked to a higher BMI, waist circumference, total cholesterol, leptin, CRP, blood pressure and reduced insulin sensitivity, indicating that PTSD is also associated with obesity-related diseases, including CVD and DM II [188]. Several mechanisms underlying how both diseases are linked to each other have been discussed in the literature. Various studies have found that PTSD and obesity are independently associated with higher rates of binge-eating, poor sleep, elevated inflammatory markers and altered levels of neuropeptides, shortened chromosome telomere length, mitochondrial dysfunction, increased endoplasmic reticulum stress and cortisol levels [189].

3.4. Dementia

Compared with men of the same age, the WHO considers older women to have lower pensions, a greater risk of poverty and poorer general health, including higher rates of dementia, combined with less access to health care and social services [3]. In particular, a low socioeconomic status appears to have a significant role in the development of dementia [190], and low socioeconomic status is also regarded as a risk factor for obesity [20].

Postmenopausal women show a decline in cognition, possibly due to the fall in oestrogen, which is considered to be neuroprotective [191] and associated with neuronal growth and certain aspects of memory [40].

Gender-specific prevalences of dementia differ along with the subtypes of dementia. While women exhibit an almost 2-fold increased risk of Alzheimer's disease (AD) compared with men, men are more susceptible to vascular dementia (VD), dementia with Lewi bodies and Parkinson's disease. However, AD contributes to the majority of cases of dementia. Risk factors for VD have been described to have a greater severity of impact on women. Gender also influences the disease course; women show a more rapid progression of AD than men once diagnosis is made [191]. In 2013, a population-based study in the UK found the prevalence of dementia to be highest in women aged ≥85 years. At the age of ≥90 years, 35% suffered from dementia. In this age group, the female brain appears to be more vulnerable to dementia, compared with in men [192]. As life expectancy increases, the number of women with dementia is growing steadily. Between 2012 and 2051, figures of dementia are anticipated to be doubled in women in the UK [193].

Dementia appears to be linked to weight in midlife. A study on twins, which included 8534 twins aged ≥65 years examined the association between BMI and dementia. Dementia was diagnosed by performing the Mini Mental State Examination. BMI at midlife, considered to be ~43 years old, was assessed by self-reporting. Dementia was found in 350 of the 8534 participants. Compared with those without dementia, twins with dementia were older, had lower levels of education, lower current BMI, and were more likely to have diabetes, cardiovascular and cerebrovascular disease. However, there was a strong link between dementia and midlife BMI; even after adjustment for age, gender, education, diabetes, hypertension, stroke and heart disease, both overweight and obesity at midlife were associated with increased risk of dementia of different subtypes, compared with a normal BMI [194]. Another large population study obtained similar findings; participants who were obese at 30–39 years of age had a significantly increased risk of later dementia, although obesity at a later age did not appear to contribute significantly to the development of dementia [195]. However, a longitudinal study involving 392 adults without dementia aged 70 at baseline reported that being overweight at age 70 was a risk factor for AD in women [143] only.

Systemic inflammation may be an important link between obesity and dementia, as cytokines may provoke neuroinflammatory processes in the brain leading to neurodegeneration. There are hints in the literature that such inflammatory dysregulation in AD may be specifically relevant in females [23, 191, 196]. In women with diabetes, the risk of developing dementia was shown to be 19% greater than in men [191].

3.5. Sleep disturbances

Insomnia is defined by difficulty in initiating or maintaining sleep, waking up too early, or sleep that is chronically non-restorative or poor in quality. These symptoms have to be associated with some daytime impairment, for example fatigue or daytime sleepiness [197]. Insomnia impacts negatively on quality of life, workplace productivity, mental health and disease morbidity [198]. A meta-analysis on gender differences in insomnia noted a greater risk in women of suffering from insomnia, with an OR of 1.4. The trend of female predisposition was consistently found among all age groups, which was higher in the elderly [199]. Another meta-analysis documented

a female-to-male ratio of 2.1 for insomnia [163]. Sleep disturbance is considered a hallmark of the menopausal transition, and insomnia affects ~50% of middle-aged women. The postmenopausal risk of OSAS is indicated to be 3.5 times greater than in premenopausal women, independently of BMI [182].

In China, a study of 24,027 men and 33,677 women aged 30–79 years investigated the association between sleep duration and DM II. They found that short (≤5 h) and long sleep durations (≥10 h) were significantly associated with DM II in postmenopausal women, but not in premenopausal women or men [200]. A similar result was reported by the National Health Service among women in the UK, with a U-shaped association between sleep duration and DM II. However, this relationship was attenuated following adjustment for BMI [113].

In addition to sleeping problems appearing to favour the development of obesity, obesity also give rise to sleep disturbances. Sleep restriction has been demonstrated to lead to higher food intake, poor food choices and unhealthy eating behaviour and, therefore, may encourage weight gain and obesity [201, 202]. Additionally, prolonged sleep duration (>8 h/night) was described as a risk factor for obesity although to a lesser extent [203]. Despite excessive sleepiness, it has been reported that up to 50% of adults with OSAS also suffer from insomnia [197]. Obesity is generally accepted as predisposing to OSAS, and 70% of patients with OSAS are obese. Weight loss significantly improves OSAS, and OSAS itself independently deteriorates the comorbidities of obesity by augmenting IR, glucose and TG levels, as well as markers of inflammation, arterial stiffness and atherosclerosis [204, 205]. In general, obesity is also associated with excessive daytime sleepiness (EDS), often due to OSAS but also found independently [206]. Hypercortisolemia and the increased production of somnogenic, pro-inflammatory cytokines have also been shown to be associated with obesity-related low sleep efficiency and EDS [207]. EDS in obesity may prevent individuals from being active during the day, therefore leading to weight gain. Moreover, individuals with EDS may tend towards daytime napping, altering nocturnal sleep [208]. This may lead to a vicious circle of impaired sleep, EDS, reduced PA and changing dietary patterns, resulting in further weight gain. **Table 2** summarises the mental health issues in women with obesity.

Aspects of mental health affected by obesity	Specific consequences
Affective disorders	Depression
Anxiety disorders	Panic disorder
	Phobia
	Social anxiety
	PTSD
Neurodegenerative diseases	AD
	VD
Sleep disturbances	OSAS
	EDS

For details regarding the association between these mental health issues and obesity see Section 3 "Mental health consequences of obesity in women". Abbreviations: posttraumatic stress disorder (PTSD), Alzheimer dementia (AD), vascular dementia (VD), obstructive sleep apnoea syndrome (OSAS), excessive daytime sleepiness (EDS).

Table 2. Mental health issues in women with obesity and the specific consequences.

4. Discussion

4.1. Summary of findings

Obesity has an impact on the majority of the 10 top health issues affecting women. ER-positive cancer types, including postmenopausal breast and endometrial cancer, are highly associated with obesity as oestrogen levels rise with BMI. Other detrimental effects of excessive fat accumulation are known to be involved in elevated cancer risk and the development of other sequelae in women. Among these are a systematic inflammatory process, often combined with an imbalance in AT deriving cytokines, disturbances in metabolic homoeostasis, alteration in the composition of the gut microbiome, increased gastro-oesophageal reflux and the emergence of gallstones. Young, obese women encounter more difficulties during pregnancy, particularly in developing countries where gynaecological care is often inadequate. Obesity gives rise to reduced fecundity and is associated with PCOS. By contrast, older women are more likely to suffer from AD than male counterparts, with increased risk in the presence of obesity at midlife. The female-to-male ratio of obesity-related MS, frequently resulting in DM II, CDV and CeVD, becomes inverted following the menopause, with prevalence rates increasing more sharply in women than in men. In terms of mental health, women appear to be more negatively affected than men for almost every stress-related psychiatric disorder, with the exception of alcohol and drug abuse [163]. Correspondingly, women receive more psychopharmacological medication, often leading to weight gain. **Table 3** summarises the mechanisms linking obesity with its associated comorbidities.

4.2. Important aspects of obesity specific to women

4.2.1. Menopause

The causes, consequences and associated disorders of obesity appear to differ among women and men due to specific gender-related factors. Physiological changes during the menopause leading to visceral fat storage may represent a crucial gender-related factor for the increased prevalence of obesity-associated comorbidities after the menopause. Consideration of the menopausal transition essential as life expectancy continues to increase. Between 2000 and 2025, the number of women aged ≥50 years old will increase by 60%, and women are estimated to spend more than a third of their life beyond the menopausal transition [40, 209].

4.2.2. Socioeconomic and cultural issues

Socioeconomic status appears to affect men and women differently. Women with a poor background have higher rates of obesity and sequelae than men, whereas men with a higher socioeconomic status are more susceptible to MS than women. Work-stress was found to have a higher impact on the development of MS in women than men. In conservative societies, women are unable to perform PA in public and, in general, women appear to be less active than men. Commonly, women are under a higher societal pressure to remain thin, which may lead to women having a poorer self-image than men, followed by depressive symptoms and poor food choices. In addition, obese women are often reported to feel uncomfortable in

Area of concern	Specific factors linking obesity to health issues in women
Socioeconomic factors	Low socioeconomic status
	Low educational level
	Ideal of thinness
	Cultural restrictions
Psychosocial factors	Probability of marriage ↓
	Shame
	Sleep disturbances
	Quality of life ↓
	Violence against women
Behavioural factors	Physical activity ↓
	Unhealthy nutrition
	Frequent dieting
	Binge-eating
	Avoidance of preventive medical screenings
Factors associated with ageing	Menopause
	Changing hormonal status (oestrogen ↓, SHBG ↓)
	Fat redistribution
Microbiome	Dysbiosis of microbiome
	TLR4 activation
Gastrointestinal system	Intraabdominal pressure ↑
	Gastro-oesophageal reflux
	Gallstones
Vascular system	RR ↑
	Atherosclerosis
Intracellular pathophysiology	Reactive oxygen species ↑
	Endoplasmic reticulum stress ↑
	Mitochondrial dysfunction
Metabolism	Glucose ↑, IGT
	Triglycerides ↑
Hormones	Oestrogen ↑
	Insulin ↑, insulin sensitivity ↓
	Cortisol ↑
	IGF-1 ↑
	Changes in neuropeptides
Adipocytokines	Adiponectin ↓
	Leptin ↑
	IL-6 ↑
	TNF-α ↑
Problems associated with medical measures	Treatment effects ↓
	Complications of surgery and radiation ↑
	Inappropriate prescription of medication
	Medication-induced weight gain
	MHT

For details see text. Abbreviations: increase (↑), decrease (↓), sex-hormone binding globulin (SHBG), Toll-like receptor 4 (TLR4), blood pressure (RR), interleukin (IL)-6, tumour necrosis factor-alpha (TNF-α), impaired glucose tolerance (IGT), postmenopausal hormone therapy (MHT).

Table 3. Mechanisms linking obesity to associated physical and mental health issues in women.

gynaecological screenings and tend to avoid these, which may result in higher mortality rates for cervical cancer in obese women.

4.3. Obesity and its consequences: the problem of causality

The direction of causality is often unclear. Obesity can be the cause of associated diseases and issues, however it can also be a sequelae of another disorder. Moreover, obesity and associated comorbidities may share causal factors. For example, obesity can lead to an increase in pro-inflammatory cytokine production, which is a risk factor for depression; depression, in turn, can lead to decreased PA and to the intake of weight-inducing psychopharmacological agents, finally contributing to the development of obesity. Additionally, sleep disturbances with consecutive EDS may be a causal factor for the development of obesity and depression. Therefore, it appears to be more appropriate to discuss "associated disorders" or "comorbidities", rather than "consequences" of obesity as, in many cases, the chain of causality is not well established.

Similarly, the mechanisms linking obesity to its associated diseases and problems are not specific. For example, an increase in pro-inflammatory cytokine production in the AT can contribute to the development of cancer, and metabolic and vascular disorders, and can be a risk factor for depression, dementia and sleep disturbances.

Obesity itself is a disorder of multifactorial causes. In addition, its consequences are linked to obesity by several factors at different levels, including socioeconomic, psychosocial and behavioural factors, and factors associated with ageing, microbiome-related mechanisms, the gastrointestinal and the vascular system, intracellular pathophysiology, problems with metabolism, hormones, adipocytokines and problems associated with medical measures, as shown in **Table 3**.

4.4. Limitations

This review does not assess the quality of the cited studies nor weigh the importance of the various consequences against one another due to space restrictions. Therefore, this chapter provides a list of the sequelae of obesity important for women with a superficial explanation of the underlying mechanisms. Moreover, the chapter does not cover the consequences of obesity, which are equally present in men and women. Therefore, the important general health problems associated with obesity concerning the eyes, respiratory system, kidneys, skeleton and muscles have not been discussed.

5. Conclusion

To conclude, this chapter identifies the risk factors leading to obesity, which are more prevalent in women than in men. These factors include a lack of PA in sport and leisure, psychiatric

problems of depression, PTSD, sleep problems and EDS, and the fact that women receive more psychopharmacological medication, compared with men.

Cancer, reproductive and maternal impairment, MS, depression and dementia are consequences of obesity with a high prevalence in women. Therefore, treatment should incorporate gender-related strategies to appropriately combat obesity and its sequelae in women. For example, clinicians may encourage particularly obese women to participate in gynaecological screenings, even if they are reluctant for their bodies be exposed owing to poor self-image.

However, despite decades of prominent research, including large-scale and molecular studies, the prevalence of obesity and its physical and mental consequences is increasing. Obesity and its sequelae are the result of a complex network of mutual interactions, including social, cultural, psychological and biological factors. This consideration indicates that there is no easy solution for such complex problems.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Chapter 8

The Role of Mesenteric Adipose Tissue in Crohn's Disease

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Additional information is available at the end of the chapter

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Abstract

Inflammatory bowel disease (IBD) has become an increasingly frequent chronic health problem in the last few decades, particularly in developing countries. In young adults, one of the most common forms of IBD is Crohn's disease (CD). CD is a multifactorial genetic disease characterized by a transmural granulomatous inflammation that especially affects the terminal ileum and the colon. As it involves defective inflammatory pathways, the immune adaptive complex, and environmental factors, this disease has periods of remission and recurrence followed by diarrhea, abdominal pain, and malnutrition, which often lead to lumen bowel stenosis associated to multiple fistulas. In addition, the growth of mesenteric adipose tissue (MAT) near the affected intestinal area is a hallmark of CD. Evidence linking the development of mesenteric and intestinal alterations in CD is increasing. The aim of this chapter is to address adipose tissue in general, the morphological and functional differences between its compartments, the main characteristics of MAT in CD, and its possible role in the etiopathology of this immune-mediated disease.

Keywords: Crohn's disease, inflammatory bowel disease, adipose tissue, mesenteric adipose tissue, inflammation

1. Introduction

Adipose tissue was initially described as an energy storehouse. However, in the last decades, it has also come to be recognized as an endocrine organ with multiple functions. Adipose tissue is composed of adipocytes, connective and nerve tissue, fibroblasts, chondrocytes, osteocytes, myocytes, and immune system cells, which constitute the stromal vascular fraction [1–3].

Adipose tissue is very dynamic, and it is able to secrete a diverse spectrum of biologically active substances designated as adipocytokines, such as cytokines, and hormone-like proteins, such as leptin, adiponectin, and resistin. These substances exhibit endocrine or paracrine functions, and they are important in maintaining energy homeostasis [1, 3–6].

Recent studies have shown that abnormal adipose tissue expansion associated with inflammation predisposes, in turn, to obesity, cardiovascular disease, chronic kidney disease, and inflammatory bowel disease [5, 7–9].

This chapter deals with adipose tissue in general, the morphological and functional differences among its compartments, and presents the main characteristics of mesenteric adipose tissue (MAT) in Crohn's disease (CD), in addition to its possible role in the etiopathology of the disease.

2. Morphological classification of adipose tissue depots

Adipose tissue can be classified into white adipose tissue (WAT), brown adipose tissue (BAT), and the recently discovered beige adipose tissue (BAT) [2, 10].

WAT comprises the vast majority of adipose tissue in the human body. WAT presents an energy-storing property and a secretory function [3–5]. WAT is anatomically divided into distinct depots: subcutaneous and visceral fat [2, 9].

Several studies have reported morphological and functional differences between these adipose tissue compartments. Obese patients have visceral adiposity and are more prone to develop insulin resistance, which strongly correlates with metabolic syndrome. Moreover, the pattern of adipokines secreted by visceral and subcutaneous adipose tissue is different [2, 9, 11].

The importance of anatomical parameters in the regulation of WAT biology is highlighted by the fact that in obese subjects abdominal deep subcutaneous WAT expands much more than superficial subcutaneous WAT [1, 9, 12]. A further instance of this can be seen in that subcutaneous and visceral WAT contribute to cardiovascular disease, whereas femoral WAT may have an overall protective effect. Therefore, changes in biological characteristics of different depots of adipose tissue give rise to different cardiometabolic conditions [9, 13].

Indeed, the heterogeneity among different anatomical depots also appears to stem from their intrinsic diversity, including cellular developmental origin, proliferative capacity, glucose and lipid metabolism, insulin sensitivity, cytokine pattern, thermogenic ability, and vascularization [1, 2, 5].

The features of obesity lie not only in WAT expansion but also in WAT dysfunction associated with qualitative changes in its biological characteristics. In an obese condition, the ingestion of excess nutrients and energy results in hypertrophy, consequent rupturing of adipocytes, and increased local inflammatory cell accumulation, including macrophages, T cells, and altered production of adipokines. These adipose tissue changes and their systemic consequences lead to the concept of obesity as a chronic inflammatory state, and they increase the risk for

multiple chronic diseases, such as type 2 diabetes, cardiovascular disease, several types of cancer, and inflammatory bowel disease (IBD) [1, 3, 5, 9].

At a molecular level, BAT is distinguished from WAT by its expression of uncoupling protein 1 (UCP1), which is crucial to mitochondrial heat production and is involved in the maintenance of body temperature [10, 14, 15]. Neonates exhibit a considerable amount of BAT and a larger fraction of total adipose tissue mass. The thermogenic function of BAT in human neonates has not been yet well assessed due to the absence of safe experimental protocols. Probably, neonates activate a non-shivering thermogenesis (NST) in order to prevent hypothermia [14, 16]. Adult humans exhibit another mechanism to produce heat, and BAT mass seems reduced when compared with neonates. Currently, the activity of BAT in humans shows an inverse correlation to age, body mass index, and the glucose level. Adult humans possess functional BAT in the neck, supraclavicular, and axillary regions, as well as around major vessels, such as the aorta [15, 16].

Thermogenesis is a major function of BAT in rodent and adult humans. Recent advances showed that this tissue may also regulate glucose and lipid metabolism and that it plays a role in regulating energy homeostasis in humans. However, little is still known about secreted adipocytokines in vivo and their role in the regulation of energy metabolism. Studies have shown that transplanted BAT from a healthy mouse into obese-induced and leptin-deficient (ob/ob) mice improves whole-body energy metabolism, increasing insulin sensitivity and reversing preexisting obesity. These effects were accompanied by modulation in the secretion of interleukin (IL)-6, adiponectin, and others cytokines [14, 15].

Recently, another type of adipose tissue, described as beige, has been described. Exposure to cold or to β3-adrenoceptor agonist treatment stimulates WAT. This tissue expresses large amount of UCP1, which can perform a thermoregulatory function. Besides that, beige adipose tissue exists within WAT, mainly in the supraclavicular region, and may be revealed by potent exposure to cold under experimental conditions [10, 14, 15].

Classical brown adipocytes and beige cells play a critical role in the maintenance of body temperature in a cold environment. Therefore, brown and beige adipocytes are promising targets for the treatment of obesity and its related metabolic disorders [10, 14, 15].

Progress in understanding the morphological characteristics of adipocytes, as well as understanding how immune cells contribute to the control of the immunometabolism can provide new potential targets of intervention. The formation of heat-producing beige adipocytes in WAT and the polarization of macrophages transitioning from an inflammatory phenotype toward an anti-inflammatory one are examples of potential targets to explore [5, 10, 14, 15].

Several studies have come out recently concerning the typically increased mesentery in CD (named "creeping fat") near the affected intestinal area. Histological characteristics of the MAT in CD with reduced adipocyte size independent of the weight body have also been reported [17, 18]. However, humoral and cellular changes in this adipose tissue are specific and differ from those observed in hypertrophied fat tissue of obese patients. The functional impact of MAT on CD development and progression is not clear yet and has been studied intensively in recent years.

3. Characteristics of mesenteric adipose tissue and its possible role in Crohn's disease etiopathology

IBD has become an increasingly widespread chronic health problem throughout the last few decades, particularly in developing countries. In young adults, one of the most common forms of IBD is CD. CD is a multifactorial genetic disease characterized by transmural granulomatous inflammation, which affects mainly the terminal ileum and the colon. Defective inflammatory pathways, immune adaptive complex, and environmental factors are involved in CD. This disease presents periods of remission and recurrence followed by diarrhea, abdominal pain, and malnutrition, which often lead to lumen bowel stenosis associated to multiple fistulas [19].

Evidence linking the development of mesenteric and intestinal alterations in CD is increasing. It has been suggested that increased visceral adiposity (of which mesenteric fat is the main component) is pathognomonic of CD. The involvement of MAT is increasingly thought to provide a mechanistic contribution to CD progression. In addition, the increased MAT near the affected intestinal area is considered a hallmark of an active and more aggressive CD [20–22].

For over a century, mesenteric anatomy has been universally depicted in an inaccurate manner. Recent observations confirm a simpler and continuous structure from the duodenojejunal flexure to the mesorectum [23, 24]. In a prospective observational study of a cohort submitted to total excisional surgery of the mesocolon, it was demonstrated that the mesentery binds in all intestinal segments [25, 26]. The mesentery is located between the intestines and the abdominal wall, although the greatest mass of MAT is present in the ileocecal region [23].

This ectopic inflamed tissue in CD patients, also referred as "creeping fat," was already identified by Crohn and collaborators in 1932 [27]. He described its thickening and suggested its possible involvement in CD, even though direct evidence was still lacking. MAT from CD patients presents a large phenotypic variation according to what is observed in surgical specimens, with notorious thickening of adipose tissue near the affected intestinal area when compared to patients who do not present CD. Therefore, surgeons are familiar with the phenotypic variation of the creeping fat, and it is used as an anatomical marker to delineate the extent of active disease in CD patients (see **Figure 1**) [28, 29].

Considering the microscopic appearance of the MAT in CD, the histopathology shows immune cell infiltration, and the adipocytes are smaller (lower mean area and perimeter) than the controls, displaying an intriguing feature [17]. To investigate this morphological feature, apoptosis was studied in these tissues. Analysis by TUNEL assay showed a significantly lower number of apoptotic cells in the MAT of CD when compared to MAT of control group [18]. There was a strong positive correlation between the adipocyte size and the apoptotic index (accessed by TUNEL). In addition, immunohistochemistry for Ki-67 was performed on all MAT samples to access the proliferation rate of the adipocytes. However, no evidence of proliferation was verified in MAT from both groups [18]. In fact, proliferation of adipocytes occurs only in severe obesity, which produces an increased adipocyte count. Surprisingly, we have an interesting situation in CD, in which the tissue looks like a MAT from a severely obese patient, but the proliferation rate is zero. Whether adipocytes migrate to the affected area or mesenchymal cells differentiate to adipocytes/fibroblasts has to be further investigated.

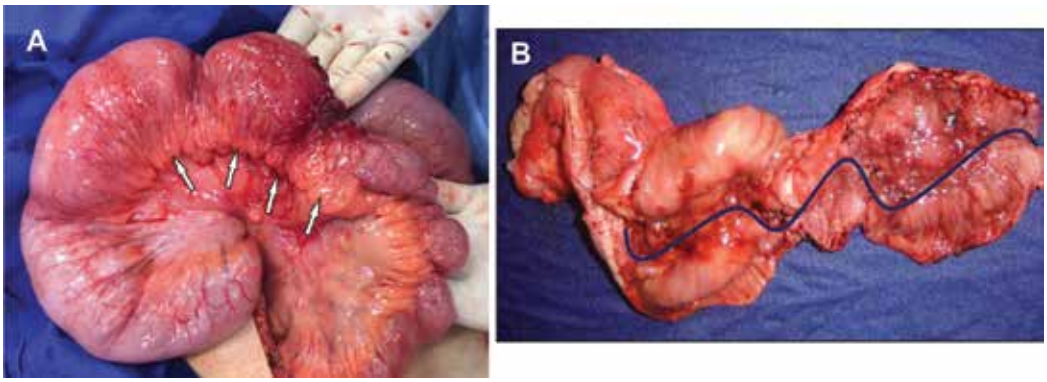

Figure 1. Surgical aspects of creeping fat (mesenteric adipose tissue) in the ileum affected by Crohn's disease. The arrows in (A) indicate the inflamed small bowel surrounded by the creeping fat. The blue line in (B) shows contiguous longitudinal ulcer in the intestinal mucosa localized in the mesenteric face of the bowel (source: Archives of Colorectal Surgery Unit—UNICAMP).

Another microscopic feature is the adipocyte hyperplasia in the submucosa of CD creating a similar histologic feature seen in the mesentery [30]. However, only colonic specimens were evaluated. This characteristic was not verified in samples from ileal CD [31]. For this reason, further investigation is needed, but fibrocytes may play a role in this, for they are increased in the mesentery of CD [32].

Indeed, MAT may present a role in the etiopathology of CD. As it has been demonstrated with other fat deposits, MAT is able to propagate both metabolic and inflammatory signals systemically, potentially modulating clinical features of CD. Its location allows MAT to respond to environmental stimuli and to coordinate intestinal responses locally and systemically [33–35].

The importance of various mesenteric components in the development and maintenance of CD, such as blood vessels, lymph nodes, and nerves, is reported [24, 26, 36, 37]. Although this relationship has not been completely elucidated, some studies provide evidence for the MAT component role in CD, as will be highlighted in the following paragraphs.

A recent study showed the presence of fibrosis, inflamed perivascular, thickened lymphatic vessels, infiltration of stromal cell, perineuronal chronic inflammation, engorgement of vasa recta, and small-sized adipocytes of MAT in patients with active CD [38]. MAT is divided into avascular and vascular regions. Within the vascularized segments, the fibro-adipose tissue involves large vessels and their ramifications [24, 39]. In healthy individuals, the mesentery is soft, and it can be easily separated from the vascularized area. However, when affected by CD, the mesentery thickens, and it interferes in the surgical dissection [28, 29].

Besides these features, histopathological findings, such as vascular lesions, focal arteritis, fibrin deposition, arterial occlusion, and granulomatous vasculitis, are observed in CD patients. These alterations demonstrate the role of mesenteric blood vessels in chronic intestinal inflammation [38, 40, 41]. Changes in vascular endothelium and abnormal leukocyte recruitment were also verified in CD [42]. Moreover, increase in the microvascular density and dysregulated angiogenic activities are other morphological and functional findings in the gut of CD patients [43].

Concerning immune cells, Kredel and collaborators identified an increase of regulatory M2 macrophages in the mesentery of CD, which suggests a protective role of the mesentery in this disease [44].

Zuo and collaborators demonstrated that the function and morphology of the normal MAT in CD patients were similar to control tissues [45]. However, increased MAT adjacent to involved ileum in CD was dysfunctional, exhibiting higher expression of hypoxia-inducible factor 1α when compared to controls, which suggests hypoxia in this tissue.

Moreover, the mesenteric nerves may also influence the pathogenesis, behavior, and prognosis of CD. In an experimental study, functional and structural alterations were observed in the mesenteric nerves of animals with colitis. Mice with colitis induced by trinitrobenzene sulfonic acid or acetic acid presented hyperexcitability of visceromotor neurons causing changes in the lower mesenteric ganglia during the intestinal inflammatory process [38, 46].

4. Molecular characteristics of the mesenteric adipose tissue in Crohn's disease

Recently, studies have highlighted a new function for MAT as an immune organ [47]. MAT's basic cellular components comprise adipocytes, preadipocytes, fibroblasts, mesenchymal stem cells, and endothelial cells. However, when the inflammatory process is initiated, an increase of immune effector cells occurs, including T cells, natural killer cells, and macrophages, as well as innate immune cells [48]. These cells are responsible for the production of several pro- and anti-inflammatory cytokines, such as TNF-α, IL-6, IL-8, IL-23, and IL-10 [49]. Given the potential therapeutic effect of their blockade, a large number of these inflammatory mediators present in MAT and their role in the development of CD have been closely investigated [31].

One of the main pro-inflammatory cytokines in CD is TNF-α. TNF-α has a fundamental role in CD due to the discovery of the therapeutic effect of its blockage [50]. The complete mechanism by which TNF-α regulates MAT inflammation in CD patients has not yet been elucidated, but it is likely a complex and multifactorial process. However, it is known that TNF-α inhibits the proliferation of new adipocytes, which leads to an increased amount of free fatty acids [51].

Another important molecule in the inflammatory process is the peroxisome proliferator-activated receptor (PPAR) transcription factor, which plays an essential role in the regulation of cellular differentiation, development, and metabolism. In CD, it has some specific functions related to the maintenance of the process, such as cytokine production, adipocyte differentiation, fibrocytes differentiation inhibition, and IκB/NF-κB activation [33, 52, 53]. On the one hand, studies have shown a decrease of the PPAR-γ in MAT from CD patients [53, 54]. On the other hand, Desreumaux and collaborators showed that significant accumulation of intra-abdominal fat is associated with overexpression of $PPAR_{\gamma}$ and TNF-α in the MAT of the small bowel mesentery in CD patients, which suggests adipocytes as one of the sources of TNF-α production [55].

Although adipose tissue is able to increase TNF-α secretion via leptin, a hormone produced by adipocytes [56], some studies in the literature did not find high levels of TNF-α in the MAT of CD, neither higher levels of IL-1B, IL6, IL8, IL23, and NF-KB activation [21, 31, 38, 57]. Again, on the

one hand, a decrease NF-kB pathway activation (decreased pIKB/IKB ratio) and increased IL-10 expression in MAT of CD patients have been demonstrated, which suggest a possible anti-inflammatory role of MAT [21, 31]. On the other hand, STAT-1 transcription factor is activated in the MAT of CD patients, suggesting a potential role in the inflammatory process [31]. This topic needs further investigation due these conflicting findings. **Figure 2** summarizes this differential expression of molecular pathways in the MAT and the intestinal mucosa of CD.

In another study, adiponectin, an anti-inflammatory adipokine, was produced and released by adipose tissue that inhibited NF-κB activation in endothelial cells in MAT of CD patients [58]. However, Rodrigues and collaborators obtained low levels of serum and mesenteric adiponectin in MAT of CD, which suggests a defect in this anti-inflammatory pathway, which could in turn help to perpetuate a state of chronic inflammation [17]. Interestingly, all patients had the same BMI, below 25, and this alteration was independent of this parameter and of the presence of metabolic disorders. Resistin, another adipokine, was also studied and was verified to be increased in MAT of CD. Resistin may have a pro-inflammatory role in the MAT and is correlated with increased systemic C-reactive protein in CD patients [59].

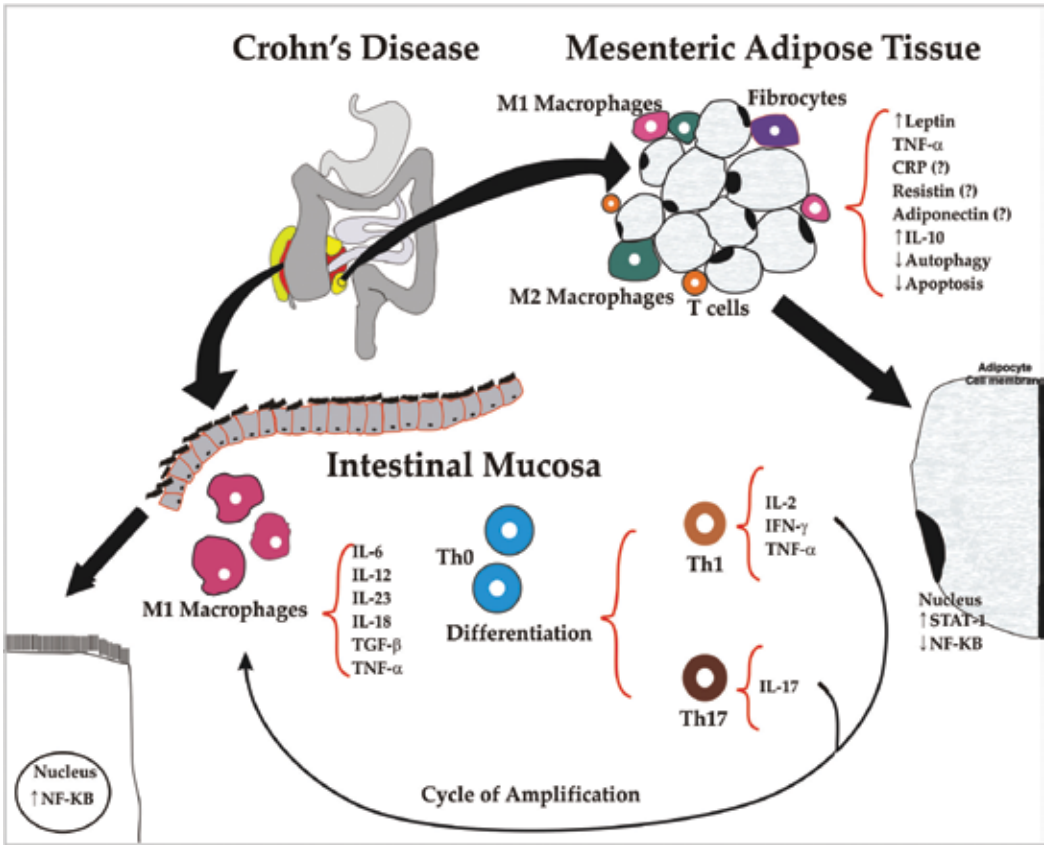

Figure 2. Molecular pathways in the mesenteric adipose tissue and intestinal mucosa of Crohn's disease patients (source: By Support Didactic from the School of Medical Sciences—UNICAMP).

Moreover, the autophagy pathways have been addressed in the last few years; for this process, it is crucial for mucosal immunity, and there is a growing number of autophagy-related genes associated with the development of CD [60–62]. Leal and collaborators demonstrated a reduction in autophagy markers in MAT of CD patients, which may maintain the inflammatory response in the affected intestine [63]. In this study, LC3-II protein, which is indispensable for the formation of autophagosome, was lower in the MAT of CD, suggesting an impairment of the autophagy process in this tissue. The altered autophagy could lead to unprocessed unnecessary protein accumulation, which activates pro-inflammatory pathways implicated in the pathogenesis of CD.

5. Conclusions

MAT may have an important role in CD inflammation, for it was possible to observe an altered balance between pro-inflammatory and anti-inflammatory factors in this tissue, decreased apoptosis, as well as defective autophagy. Available data indicate that mesenteric changes are primarily anti-inflammatory but can ultimately cause inflammation in CD. Currently, the mesenteric events in the chronology of CD are under discussion. MAT may be involved in the maintenance of inflammation in the late stages of the disease and in the mechanism that leads to relapses during the course of the disease. Moreover, the interaction between cytokines, adipokines, transcription factors, adipose stem cells, vascular endothelia, and adipocyte plasticity may imply in MAT remodeling, which certainly influences CD physiopathology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Section 4

Adipose Tissue as an Endocrine Organ

Chapter 9

The Heterogeneity of White Adipose Tissue

Quyen Luong and Kevin Y. Lee

Additional information is available at the end of the chapter

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Abstract

The increasing prevalence of obesity is a major factor driving the worldwide epidemic of type 2 diabetes and metabolic syndrome. Adipose tissue not only stores energy, but also controls metabolism through secretion of hormones, cytokines, proteins, and microRNAs that affect the function of cells and tissues throughout the body. Accumulation of visceral white adipose tissue (WAT) leads to central obesity and is associated with insulin resistance and increased risk of metabolic disease, whereas accumulation of subcutaneous WAT leads to peripheral obesity and may be protective of metabolic syndrome. While much attention has been paid to identifying differences between white, brown and brite/beige adipocytes, there is growing evidence that there is functional heterogeneity among white adipocytes themselves. This heterogeneity, includes depot-specific differences in development, inflammation, and endocrine properties. In addition to the depot-specific differences, even within a single fat depot, WAT is composed of developmentally and phenotypically distinct subpopulations of adipocytes. The following chapter will introduce this concept of white adipocyte heterogeneity.

Keywords: heterogeneity, subpopulations, inflammation, microRNA, and adipokine

1. Introduction

The prevalence of obesity, characterized by excess of adipose tissue, has been increasing worldwide and represents one of the most significant public health problems of our time. Obesity is associated with numerous comorbidities, including type 2 diabetes, coronary heart disease, hypertension, hepatosteatosis, and even cancer. Adipose tissue is organized in discrete depots in specific locations throughout the body. This chapter will briefly introduce the two major types of fat, brown and white. We will introduce the major different WAT depots and more fully elaborate the physiology of two more recently defined depots: the dermal and

bone marrow adipose tissue. We will then focus on visceral and subcutaneous white adipose tissue and discuss the differential developmental, inflammatory, and endocrine properties of these depots. The depot-specific expression and roles of inflammatory cytokines, adipokines, and novel signaling molecules, including lipokines and microRNAs will be discussed. Finally, we will discuss emerging literature that demonstrate WAT is composed of developmentally and phenotypically distinct subpopulations of adipocytes.

2. White, brown, and brite adipose tissue

The two major forms of adipose tissue include white adipose tissue (WAT) or brown adipose tissue (BAT). Although these tissues are characterized by lipid accumulation, these two tissues differ dramatically in morphology, developmental lineage, and function. WAT, is characterized by adipocytes with large unilocular droplets and is present in far greater amounts than BAT. WAT acts as the primary reserve for surplus energy in the body, storing excess nutrients as triacylglycerol (TAG). In contrast, the brown adipocytes actively dissipate energy through the production of heat. Brown adipocytes contain multilocular lipid droplets distributed throughout the cell. Brown adipocytes contain more mitochondria than white adipocytes, which, along with an increased capillary density, is responsible for the brown color of BAT [1]. In the unique thermogenic property of brown fat is due to the presence of uncoupling protein-1 (UCP1). UCP1 allows the reentry of protons pumped across the inner mitochondrial membrane by respiratory chain enzymes. This converts the energy of the mitochondrial proton gradient into heat. The importance of UCP1 to brown fat function is evident in studies of mice with targeted UCP1 ablation, which results in cold intolerance, with variable effects on WAT accumulation and obesity [2, 3].

The identification of a third adipocyte type, termed "brown-in-white", "brite", or "beige" that has many of the functional characteristics of BAT while being dispersed throughout WAT depots. Like its BAT, beige fat has the capacity for thermogenesis, expresses UCP1, and can be activated in response to cold exposure or adrenergic stimulation [4]. Although brown adipocytes are largely derived from Myf5-expressing expressing lineage, evidence exists that beige adipocytes are formed from both transdifferentiation of unilocular white adipocytes and from a unique Myf5 negative precursor population within subcutaneous depots [5, 6]. However, more recent evidence suggests the presence of functionally distinct populations of beige adipocytes [7] that are molecularly distinct from brown and white adipocytes in both mice and humans [8, 9]. Since the discovery that most humans possess active BAT, primarily in the supraclavicular regions [10–13], increasing the amount and activation of both BAT and beige AT to combat obesity has been an extremely active avenue of research.

3. White adipose tissue depots

WAT serves multitude of functions including storage of lipid, maintenance of insulin sensitivity, and endocrine signaling [14]. Adipocytes in WAT are characterized by low cytoplasmic volume, unilocular lipid droplets, and lower numbers of mitochondria compared to BAT. WAT can be categorized into two major subdivisions based on the anatomical locations

or depots: subcutaneous (fat under the skin in the hypodermis region) and visceral. Increase in visceral fat is related to the increased risk of metabolic disorders such as type 2 diabetes and cardiovascular diseases [15, 16], whereas subcutaneous fat is not and may even be protective against metabolic derangements [17]. The differences between these two types of WAT are attributable to both intrinsic differences in the cells that comprise these depots as well as differences in the micro-environment between adipose tissue depots.

3.1. Subcutaneous adipose tissue

In rodents, subcutaneous WAT is divided into subcutaneous anterior fat (SAF) and subcutaneous posterior fat (SPF). SAF can be further subdivided into cervical, axillary, interscapular, and subscapular, and SPF is divided into dorsolumbar, inguinal, and gluteal [18]. In humans, two subcutaneous fat regions are also recognized: upper and lower body fat, where they correspond approximately to SAF and SPF, respectively. Upper body subcutaneous fat consists of superficial and deep layers separated by the Scarpa's fascia. Superficial fat is compact, consistent in thickness, and metabolically less active compared to deep layer fat [19]. Lower body subcutaneous fat is primarily made up of adipose tissue around the gluteal and femoral (gluteofemoral) regions [20, 21]. Accumulation of gluteofemoral fat is associated with improved glucose tolerance [22], negatively correlated with insulin resistance [17], and associated with reduced aortic calcification related to cardiovascular diseases [23]. However, the protective effect of abdominal subcutaneous fat is disputed, potentially as a result of the presence of deep subcutaneous fat, which has been suggested to behave similar to visceral fat regarding metabolic parameters such as insulin-stimulated glucose utilization [24]. There has been no evidence of multiple subcutaneous AT layers in rodents, such as is the case in humans.

3.2. Visceral adipose tissue

Visceral fat is generally regarded as intra-abdominal adipose tissue that surrounds internal organs. Under this definition, the major human visceral depots are: the omental, retroperitoneal, perirenal, mesenteric, and pericardial depots [18, 20]. Notably, only the mesenteric and omental adipose tissues drain directly into the portal circulation, and thus release of free fatty acids (FFAs) and pro-inflammatory cytokines from these depots is directly delivered to the liver and promotes the development of hepatic steatosis and insulin resistance [21, 25]. Mice have similar visceral adipose tissues to humans including the mesenteric, perirenal, pericardial, and retroperitoneal fat depots. However, rodents have a well-developed perigonadal fat pad, which is largely absent in humans, while rodents have a paucity of omental adipose tissue (**Table 1**).

The enlargement of visceral adipose tissue is largely detrimental to the functions of the surrounding organs. Pericardial fat, including both epicardial and pericardial AT, is associated with metabolic disorders and low-grade inflammation, resulting in type 2 diabetes and cardiac complications. Increase thickness of pericardial AT is associated with the increase of diastolic pressure and fasting insulin [26, 27], arterial calcium [28], and severity of coronary artery disease [29]. Similarly, an increase in perirenal (fat between renal fascia and capsule) and pararenal AT (immediately external to renal fascia) thickness is correlated with glomerulopathy [30], increased frequency of chronic kidney disease in type 2 diabetic patients [31], and hypertension due to compression of low-pressure structures in the renal sinus such as veins,

	Subcutaneous	Visceral	Other
Humans	Upper body: superficial and deep abdominal (separated by Scarpa's fascia)	Omental, retroperitoneal, perirenal, mesenteric, pericardial	Bone marrow, dermal
	Lower body: gluteofemoral (butt and thigh)		
Rodents	Anterior: cervical, axillary, interscapular, subscapular	Perigonadal, perirenal, pericardial, mesenteric, retroperitoneal	Bone marrow, dermal
	Posterior: dorsolumbar, inguinal and gluteal		

Table 1. Major adipose depots in humans and mice.

lymphatic vessels, and ureters [32, 33]. Increased mesenteric fat is associated with increased risks of cardiovascular diseases [34], Crohn's disease [35], and hepatic insulin resistance and hepatosteatosis [36]. Together, these studies show that increased visceral, but not subcutaneous fat deposition, is associated with numerous disease states and metabolic derangements.

3.3. Other white adipose tissues

3.3.1. Dermal white adipose tissue (dWAT)

Recent research has drawn attention to a newly recognized adipose depot, the dermal white adipose tissue (dWAT) [37]. dWAT is the widespread adipose tissue found in the reticular region of the dermis, and in mice is separated from the subcutaneous adipose tissue by a striated muscle layer. In mice, evidence suggests that adipocytes from dWAT develop independently from subcutaneous depot [38]. On the other hand, human dWAT is not clearly separated from the underling subcutaneous depot and is defined by dermal cones that concentrate around hair follicles [39]. Clusters of dWAT are more densely distributed in areas that are highly-prone to scaring [40]. In fact, dWAT is now known to be associated with numerous functions including scar formation, wound healing, and cutaneous fibrosis [41–45]. The wound healing mechanism involves inflammatory response and closing of the area by fibroblast migration, which the latter is mediated by adipocyte activation. This process is characterized by an intra-conversion between adipocytes and myofibroblasts and also contributes to the fibrosis observed in scar formation and autoimmune diseases (i.e. scleroderma) [37, 46, 47].

In addition to wound healing effect, dWAT plays an important role in hair follicle cycling. Preadipocytes, but not mature adipocytes in the dWAT have been suggested to activate the growth of hair follicles [48, 49]. As dWAT develops independently from subcutaneous depot, its emergence in embryonic stage coincides with the development of hair follicles, at least in murine fetuses [38], further supporting the relationship between dWAT and hair follicle development.

Dermal adipose tissue has also been suggested to function in other processes including protection of skin from bacterial infection and whole-body thermoregulation. Infection with *S. aureus* promotes rapid proliferation of pre-adipocytes, leading to large expansion of dWAT and increased production of antimicrobial cathelicidin [50], suggested a protective response of dWAT to bacterial infection. Loss of syndecan-1, an important adipocyte differentiation protein, leads to reduced thermoregulation and loss of dWAT, implying a role of dWAT in regulating temperature [51].

3.3.2. Bone marrow adipose tissue (BMAT)

Bone marrow adipose tissue (BMAT) is, as the name suggests, is located within the bone marrow. Bone marrow adipocytes are known to share common origin with osteocytes, chondrocytes and hematopoietic cells, as indicated by lineage tracing models [52]. As a fat depot, BMAT makes up 10% of human fat mass and up to 70% volume of bone marrow [53]. The BMAT adipocytes consist of two types in mice: constitutive bone marrow adipocytes (cBMA) and regulated BMA (rBMA) [54]. cBMAs are large adipocytes that densely populate regions of distal tibia and caudal vertebrae. These adipocytes develop early in life, contain high levels of unsaturated fatty acids, and are resistant to insulin and beta-adrenergic stimuli. On the other hand, rBMAs are distributed across the trabecular regions of proximal tibia, distal femur, and lumbar vertebrae. These adipocytes are smaller and have higher saturated fat than cBMAs and subcutaneous adipocytes [55]. Additionally, rBMAs respond to beta-adrenergic stimuli and dietary changes [54]. Interestingly, BMAs exhibit characteristics of both WAT and BAT and express both WAT and BAT markers. BMAs express adipogenic markers and resemble WAT in terms of the unilocular appearance and the capability to secrete adiponectin and leptin [56, 57]. However, like BAT or brite adipocytes, the distribution of these cells are dependent on temperature and location within the body [54, 58]. The BAT characteristics of BMAT decrease with age and in pathological condition such as diabetes [59].

Numerous physiological and pathological processes influence BMAT physiology. BMAT expansion occurs in normal aging, primarily due to an increase in rBMA over time [54, 60]. Expansion of BMAT and reduction of bone volume are observed in human subjects with osteoporosis [61]. Steroid hormones also modulate BMAT expansion, as both estrogen deficiency [62, 63] and excess glucocorticoids, observed in Cushing's disease, have also been shown to increase BMAT [64, 65]. On the other hand, in a location and subtype dependent manner, leptin potentially antagonizes adipogenesis in bone marrow as observed in both caloric restriction and leptin-deficiency [66–70]. Furthermore, high-fat diet (HFD) causes BMAT expansion and bone loss [71–73]. Treatment of type 2 diabetes with thiazolidinedione (TZD) increases BMAT mass. Although the relationship between increased BMAT and reduced cortical and trabecular bone mass remain controversial, these studies could possibly discourage TZD administration to patients with high risk of bone fracture [72, 74–77].

The physiological functions of BMAT in normal and pathological conditions are beginning to be explored. Inflammatory cytokines have been found to be secreted by BMAT and the secretion of these molecules may be altered by diet induced obesity [71, 78, 79]. Bone marrow adipocytes have also been shown to produce adiponectin. Particularly during caloric restricted state and anorexia nervosa during which all adipose tissues except BMAT are depleted, BMAT is a major source of circulating adiponectin [53, 80–83]. Additionally, BMAT influences hematopoiesis and osteogenesis in the marrow environment. BMAT has been shown to negatively regulate hematopoiesis [84] and bone marrow adipocytes may also play a role in bone remodeling. Increased bone marrow adipocytes leads to the increased expression of RANKL, which induces the activity of osteoclasts and reduces bone density [85]. Similarly, osteoporosis is accompanied by a marked increase BMAT mass [86]. Future studies will add to our understanding of the regulation and physiological contribution of BMAT.

4. Intrinsic differences between visceral and subcutaneous adipocytes

Recent lineage tracing studies have indicated that visceral and subcutaneous WAT are derived from different developmental lineages [87]. This finding supports earlier findings that preadipocytes and adipocytes from these depot have intrinsic depot-specific differences in both gene expression and function.

In general, preadipocytes derived from subcutaneous regions are more pro-adipogenic and readily differentiate into adipocytes, whereas visceral preadipocytes express anti-adipogenic genes and require additional components for differentiation [88–91]. The increased differentiation of subcutaneous-derived preadipocytes may due, at least in part, to high levels of expression of pro-adipogenic genes, PPARy and C/EBPs coupled with the high number of rapidly replicating preadipocytes derived from subcutaneous tissue [92–96]. These intrinsic differences could contribute to the protective effect of subcutaneous fat during obesity, where hyperplasia in subcutaneous fat allows the uptake of excess fat and prevents ectopic deposition. On the contrary, visceral fat has lower lipoprotein lipase activity and higher rates of catecholamine-induced lipolysis. This leads to an increase in free fatty acid release from visceral adipose tissue into the portal circulation [97–100]. These differences in gene expression, differentiation, and replication are retained after numerous passages of cultured subcutaneous and visceral preadipocytes, thus revealing intrinsic, cell-autonomous differences which contribute to the regional differences in mature adipocytes.

In addition to the large differences between visceral and subcutaneous adipocytes, inter-depot differences also exist even with subcutaneous and visceral adipose tissue. Within subcutaneous depot, abdominal preadipocytes express higher pro-adipogenic marker PPARγ, are more susceptible to apoptosis upon inflammatory cytokine exposure, and are smaller in size due to increased lipolysis compared to gluteofemoral subcutaneous fat [90, 99, 101]. Similarly, not all visceral adipose tissues are the same. Mesenteric adipocytes are intermediate between abdominal subcutaneous and omental in terms of replication and differentiation [92, 93, 95, 96]. Furthermore, the perirenal depot contains a higher percentage of rapidly dividing cells than perigonadal fat [96, 102–104]. Together, these studies demonstrate that variations in subcutaneous and visceral depots are dependent not only on anatomical location, but also upon the intrinsic properties of the adipocytes found within the depots.

5. Associations of WAT depots with metabolic health

As previously mentioned, accumulation of visceral fat, termed central obesity, is associated with increased risk of diabetes, and cardiovascular diseases [23, 105–107] while subcutaneous fat has been linked to protection from metabolic diseases [17, 22, 108]. The differential effects of subcutaneous and visceral adipose tissue on metabolism have been directly tested by transplantation and surgical removal of adipose tissue. While transplanting subcutaneous adipose tissue improved the glucose tolerance of the recipient animals, transplantation of visceral fat

did not have this effect [109, 110]. Similarly, removal of visceral fat restores insulin sensitivity in rats and in humans, but removal of subcutaneous did not improve metabolic profiles [111–113]. Thus, visceral WAT is strongly associated with metabolic syndrome. The following section of this chapter will discuss the depot-specific regulation of inflammation, immune cells, and cytokines and how these factors impact whole-body physiology.

5.1. The role of immune system in obesity-related metabolic syndrome

Macrophages have an established role in regulating angiogenesis during tissue repair [114]. In the early expansion of obese adipose tissue, remodeling of extracellular matrix occurs along with increased angiogenesis to support growing adipocytes [115, 116]. However, continued hypertrophy of adipocytes in later stage of obesity leads to reduced oxygen tension, and expression of hypoxia-inducible factor 1α (HIF1α) is induced in the adipose tissue. HIF1α has been shown to be elevated in obese mice and humans [117–120]. Increased HIF1α is associated with the development of fibrosis, inflammation, and insulin resistance [119, 121–123].

The negative impacts of visceral fat depots on metabolism are, at least in part, attributable to the macrophage infiltration and inflammation that occur primarily in the visceral adipose tissue. The immune system plays an intricate role alongside of adipose dysfunction during the development of obesity-related metabolic syndrome. Obesity-induced metabolic disease is now classified as a chronic-inflammatory disease due to the presence of immune cells and elevated levels of inflammatory cytokines. In lean mice and humans, low levels of macrophages are found in adipose tissue. However, obese mice and human subjects have an increased number of macrophages, especially in the visceral adipose tissue, with numbers correlating with the increased size of adipocytes and body fat mass [124, 125]. The Infiltrating macrophages in obesity are polarized towards a classically activated M1 pro-inflammatory phenotype and surround dying adipocytes in the form of multinucleated giant cells and crown-like structures [126, 127]. The number of alternatively-activated M2 macrophage number does not change during obesity but is overwhelmed by the increased presence of recruited M1 macrophages, leading to an overall shift in the ratio of these macrophages [128].

Macrophage recruitment relies on chemoattractant proteins, such as monocyte chemoattractant protein (MCP)-1 or chemokine (C-C motif) ligand 2 (CCL2). The initial dose of MCP-1 release was found to be secreted by pre-adipocytes [129], supporting the hypothesis that initial recruitment of macrophages is necessary for extracellular matrix remodeling and tissue expansion. Post-recruitment, macrophages are activated by other immune cells, in particular cytotoxic cells, initiating an inflammatory cascade. Adipose CD8 cytotoxic T cells that normally kill virus-infected cells are activated by obese adipocytes, which leads to subsequent activation and M1 polarization of macrophages. This macrophage polarization event precedes macrophage infiltration and occurs as an early response to high-fat-diet (HFD) exposure in mice [130]. Natural killer (NK) cells, which are cytotoxic cells that participate in innate immunity, recruit and activate macrophages through secretion of MCP-1 and IFN-γ. Activated macrophages, in return, recruit via the secretion of CCL3, CCL4, and CXCL10, and stimulate the proliferation of NK cells through release of IL-15 [131]. Other immune cells, including B and different types of T cells, indirectly contribute to pro-inflammation state of

adipose tissue. B cells are important participants of humoral immunity, secrete inflammatory cytokines (IL-8, IL-6, IFN-γ), and activate both CD4 and CD8 T cells [132]. B cells support adipocyte hypertrophy and the pro-inflammatory T-cell function in obesity/T2D through cellular contact-dependent mechanisms. Thus, reducing the interaction between antigen presenting B cells and T cells decreases the inflammatory response and can lead to improvements in glucose and insulin metabolism [132, 133]. While the effects of pro-inflammatory immune cells are principal regulators of adipose tissue in the obese state, anti-inflammatory cells (i.e. M2 macrophages, regulatory T cells (Treg), and T helper type 2 cells (Th2)) also have defined roles in adipose tissue homeostasis [134].

5.2. Inflammatory cytokines

The macrophage infiltration which occurs during obesity, particularly visceral adipose tissue, lead to increased local and systemically levels of inflammatory cytokines [135]. In the following section, we will discuss the regulation and action of some of the major inflammatory cytokines within adipose tissue.

5.2.1. Tumor necrosis factor-α (TNF-α)

TNF-α was the first identified cytokine derived from adipose tissue macrophages that links both obesity and inflammation. TNF-α mRNA and protein levels have been shown to be elevated during obesity in the adipose tissue both animal models and human subjects. Increased TNF-α is positively correlated with increased degree of obesity and circulating insulin level, whereas TNF-α level decreases with weight loss and increased insulin sensitivity [136–140]. These effects are directly attributable to TNF-α, as infusion of a TNF-α neutralizing antibody, or ablation of TNF-α or its receptor in mice leads to improved insulin sensitivity [140–142]. Despite these clear results in mouse models of obesity, the use of TNF-α neutralizing antibodies and inhibitors has had inconsistent success in treating insulin resistance and glucose intolerance in obese human subjects [143–146].

TNF-α affects a myriad of various pathways to alter adipose tissue metabolism. TNF-α impairs insulin signaling via downregulation of insulin receptor through phosphorylation of insulin receptor substrate-1 (IRS1) and suppresses adipogenesis by controlling the transcriptional regulation and activity of the adipogenic factors PPARy and C/EBPs [14, 147, 148]. Furthermore, TNF-α induces lipolysis through the downregulation of anti-lipolytic genes perilipin, FSP27 and G0S2 and inhibition of lipoprotein lipase activity. TNF-α can directly cause apoptosis in visceral pre-adipocytes and adipocytes [149–155]. Taken together, the actions of TNF-α function to reduce adipocyte size and number, leading to the release of free fatty acids into the circulation.

5.2.2. Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is secreted by numerous cell types including the adipocytes and macrophages, with only 10% of IL-6 being contributed by adipocytes [124, 156, 157]. Multiple lines of evidence point to visceral adipose tissue as the major contributor of circulating IL-6 [158, 159]. Like TNF-α, IL-6 also negatively regulates insulin signaling through degradation of IRS1 [148].

5.2.3. Interleukin-1 receptor antagonist (IL-1Ra)

IL-1Ra is a natural antagonist to inflammatory cytokine interleukin-1α and β. IL-1Ra is expressed in numerous tissues, and is highly expressed in adipose tissue during obesity, and its expression is positively correlated with leptin level. Indeed leptin is capable of inducing IL-1Ra; and as a negative feedback loop, IL-1Ra antagonizes leptin activity [97, 160]. Targeting IL-1Ra has intriguing therapeutic potential, as treatment of diabetic patients with a recombinant human interleukin-1-receptor antagonist increased insulin secretion from pancreatic islets [161]. Interestingly, a single nucleotide polymorphism in IL-1Ra is highly associated with body fat mass [162].

5.2.4. Plasminogen activation inhibitor-1 (PAI-1)

PAI-1 is another inflammatory cytokine more highly expressed in visceral than subcutaneous adipose tissue. In human subjects. Plasma PAI-1 level correlates with body mass index [163]. PAI-1 is expressed in mature adipocytes, monocytes, as well as other stromovascular cells from the adipose tissue [164, 165]. Ablation of PAI-1 in mice leads to improved glucose and insulin metabolism [166], and PAI-1 has been found to negatively regulate adipogenesis [167]. IL-6, but not TNF-α, stimulates PAI-1 expression in human adipose tissue [163, 165].

6. Depot-specific effects of adipokines and other signaling molecules

As an endocrine organ, WAT secretes a variety of hormones and cytokines, also known as "adipokines". While another chapter in this book will provide a broader overview of the endocrine functions of AT, we would be remiss if we did not mention the depot-dependent adipokine profile of AT. In addition, we will discuss two recently discovered classes of endocrine signaling molecules derived from adipose tissue: distinct lipid species, known as "lipokines" and circulating microRNAs.

6.1. Adipokines

Adiponectin is an adipokine that has anti-inflammatory and insulin-sensitizing action [168]. The majority of reports suggest that adiponectin secretion is primarily driven by subcutaneous rather than visceral fat, and that adiponectin level are low in obese and insulin resistant patients [97, 169–171]. Inflammatory cytokines reduce adiponectin secretion, especially in the visceral adipose tissue [169]. Not only is reduced adiponectin involved in insulin resistance, but albuminuria, a marker of kidney damage, is related to adiponectin deficiency [172], further extending the protective effects of adiponectin in metabolic health.

Leptin is a satiety hormone primarily secreted by adipocytes that acts on the hypothalamus to decrease food intake and increase energy expenditure, among other functions. As such, mice and humans with mutations of leptin or its receptor exhibit marked obesity [173–175]. Leptin is secreted by adipocytes and levels are positively correlated with the amount of body fat [135, 176]. Secretion of leptin appears to be depot-dependent, with subcutaneous WAT producing greater amounts than visceral WAT [89, 159, 170, 177, 178].

Resistin is a peptide hormone expressed in adipose tissues of both rodents and humans. In rodent, the primary source of resistin are the mature visceral adipocytes, but in humans the visceral fat macrophages are the major contributor of circulating resistin [179–182]. Anti-resistin treatment or loss of resistin signaling improves insulin sensitivity and glucose homeostasis, while recombinant resistin treatment impairs glucose and insulin metabolism [181, 183, 184]. Although the cellular source of resistin is different between humans and mice, macrophage-derived human resistin is also sufficient to exacerbate adipose tissue inflammation and insulin resistance in mice [185].

Visfatin (or pre-B cell colony enhancing factor PBEF) is an adipokine named for the suggestion that it would be predominantly produced and secreted in visceral fat [186]. Visfatin was found to be released predominantly from macrophages rather than from adipocytes in visceral adipose tissue, and plasma visfatin significantly correlates with BMI and body fat [187]. Visfatin has been shown to have endocrine, paracrine, and autocrine action, and may function through binding of the insulin receptor [186].

Retinol binding protein 4 (Rbp4) is a secreted factor from adipocyte tissue that has marked metabolic effects both on liver and skeletal muscle. Ablation of Rbp4 leads to improvements of glucose and insulin metabolism while addition of Rbp4 impairs insulin signaling in muscle [188]. Rbp4 expression is dramatically increased by obesity and insulin resistance in humans, and is much more highly expressed in visceral than subcutaneous adipose tissue [189, 190].

Apelin is an insulin-regulated adipokine expressed in mature adipocytes whose expression is increased in obesity. Apelin appears to be equally expressed in visceral and subcutaneous adipose tissue [191]. Apelin inhibits diet-induced obesity through increasing lymphatic and blood vessel integrity and enhancing brown adipogenesis [192, 193].

6.2. Lipid mediators "lipokines"

Recent studies have determined that specific lipid species communicate from adipose tissue to distal sites, and act as a new class of molecules termed "lipokines". The first lipokine, C16:1n7-palmitoleate, is derived from adipose tissue and regulates gene expression and insulin sensitivity of both muscle and liver [194]. Another class of lipokine, fatty acid esters of hydroxy fatty acids (FAHFAs) are reduced in serum and adipose tissue of insulin-resistant people and high-fat diet-fed mice. Administration of FAHFAs increases insulin-mediated glucose uptake into the liver and skeletal muscle [195, 196]. Finally, a BAT specific lipokine, 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) has also recently been identified. 12,13-diHOME is a stimulator of BAT activity and its circulating levels are negatively correlated with body-mass index and insulin sensitivity. 12,13-diHOME increases fatty acid uptake into brown adipocytes by promoting the translocation of the FA transporters to the cell membrane [197].

6.3. MicroRNAs

MicroRNAs (miRNAs) are non-coding RNAs that are ~22 nucleotides in length that regulate mRNA translation. Each miRNA can regulate multiple mRNA targets, and each mRNA target can be regulated by multiple miRNAs. Primary miRNAs are transcribed, and cleaved in

a multi-step process by ribonuclease enzymes, including Drosha and Dicer, to form mature miRNAs. The mature miRNAs are then loaded into the RNA-induced silencing complex (RISC), and are directed to the 3′ untranslated region (UTR) of the target mRNAs to modify their translation [198–200].

6.3.1. Circulating MicroRNAs as endocrine signaling molecules

Adipocyte-specific ablation of Dicer (ADicerKO) produces mice with a lipodystrophic phenotype marked by insulin resistance, dyslipidemia, and a reduction in both local and circulating miRNA (packaged within exosomes), suggesting important roles of miRNAs in adipocyte functions [201, 202]. Transplantation of wild-type adipocytes into ADicerKOs leads to improved metabolism. Notably, a depot-specific contribution of adipose tissue to the circulating exosomal miRNA transcriptome was observed. Furthermore, these adipose-derived circulating RNAs can also modify gene expression in other tissues, including the liver [203]. Likewise, exosomal transfer of macrophage-derived miRNAs can control gene expression and metabolism in adipocytes [204, 205]. Thus, like adipokines or lipokines, miRNAs can function as both paracrine and endocrine signals molecule to alter the physiology of distinct target tissues.

6.3.2. Cell autonomous actions of MicroRNAs in WAT

6.3.2.1. MicroRNA regulation of preadipocyte determination and adipogenesis

The formation of adipocytes from mesenchymal stem cells is based on inhibition of other lineages (chondrocyte, osteocyte, and myocyte) and promotion of adipocyte lineage (**Figure 1**). Runt-related transcription factor 2 (Runx2) and bone morphogenetic protein (BMP)-2, both osteogenic factors, are inhibited by adipose tissue expressed miRNAs. Chondrogenesis is controlled by TGF-β, which is regulated by miR-21, a miRNA that is known to be increased in human obesity and type 2 diabetes [206–208]. miR-148 and -124 target adipogenic inhibitors Wnt1 and Sox9, respectively, at the initiation of adipogenesis [200, 209, 210].

After committing to adipocyte lineage, lipid accumulation in differentiating adipocytes is controlled, at least in part by the expression and activity of PPAR and C/EBP proteins. miR-375 suppresses ERK1/2 phosphorylation which allows the activation of PPARγ [211]. miR-143 and -103 are both increased during adipocyte differentiation and have clear roles in lipid accumulation, especially within subcutaneous WAT, as confirmed by both over-expression and inhibition studies [212, 213]. miR-519d inhibition of PPARα reduces fatty acid oxidation and increase lipid storage [214], and reduced adipocyte size in human subjects is correlated with reduced expression of miR-519d [215]. On the other hand, miRNAs that target PPARγ including miR-27 and miR-130 act as anti-adipogenic regulators [216, 217] (**Figure 2A**).

6.3.2.2. MicroRNA regulation of adipocyte metabolism and inflammation

MicroRNAs target all aspects of adipocyte metabolism and a comprehensive examination of these effects is not possible within the confines of this chapter. However, we will briefly discuss how miRNAs directly regulate insulin signaling and modulate the inflammatory response of adipocytes. miRNAs can impair insulin signaling by targeting many of the key

molecules involved, including: effects on insulin receptor, IRS1, and GLUT4 (**Figure 2B**). Insulin receptor stability is partially dependent upon the protein caveolin-1, which itself is a target of miR-103. Inhibition of miR-103 thus increases insulin receptor stability and leads to improved insulin sensitivity [218]. IRS1 is downregulated by miR-139-5 and -144 [219, 220] while insulin-stimulated glucose uptake through GLUT4 is downregulated with high expression of miR-93 and -223 [221, 222] (**Figure 2B**). Macrophage infiltration is directed by expression of chemokine (C-C motif) ligand 2 (CCL2 or MCP-1). CCL2 expression is increased by miR-145, but is reduced by miR-126 and miR-193b [223]. miRNAs also control polarization of classically activated pro-inflammatory (M1) macrophages and alternatively activated anti-inflammatory (M2) macrophages. Increasing miR-223 reduces expression the

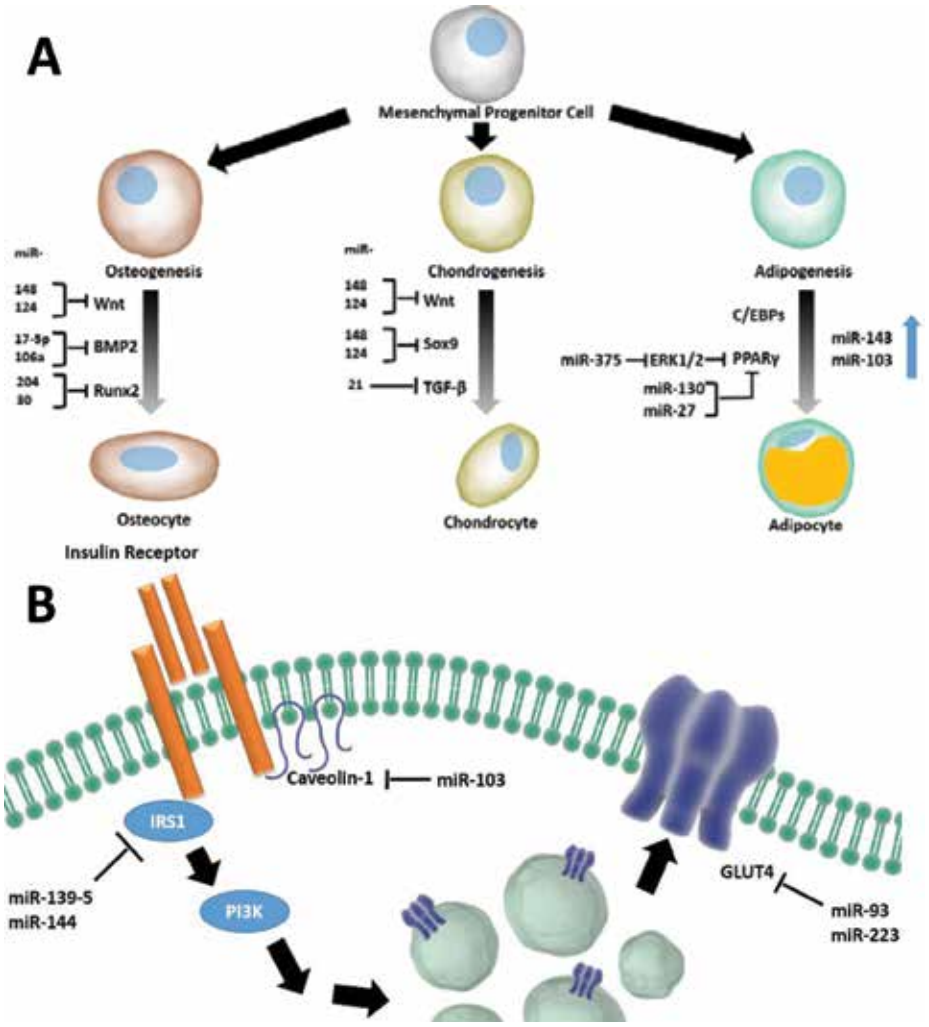

Figure 1. The roles of microRNAs in adipogenesis and insulin signaling. (A) miRNAs play an important role in promoting adipogenesis and inhibiting osteogenesis and chondrogenesis. (B) miRNAs participate in insulin resistance by targeting IRS1, insulin receptor stabilizer (caveolin-1), and GLUT4 expressions.

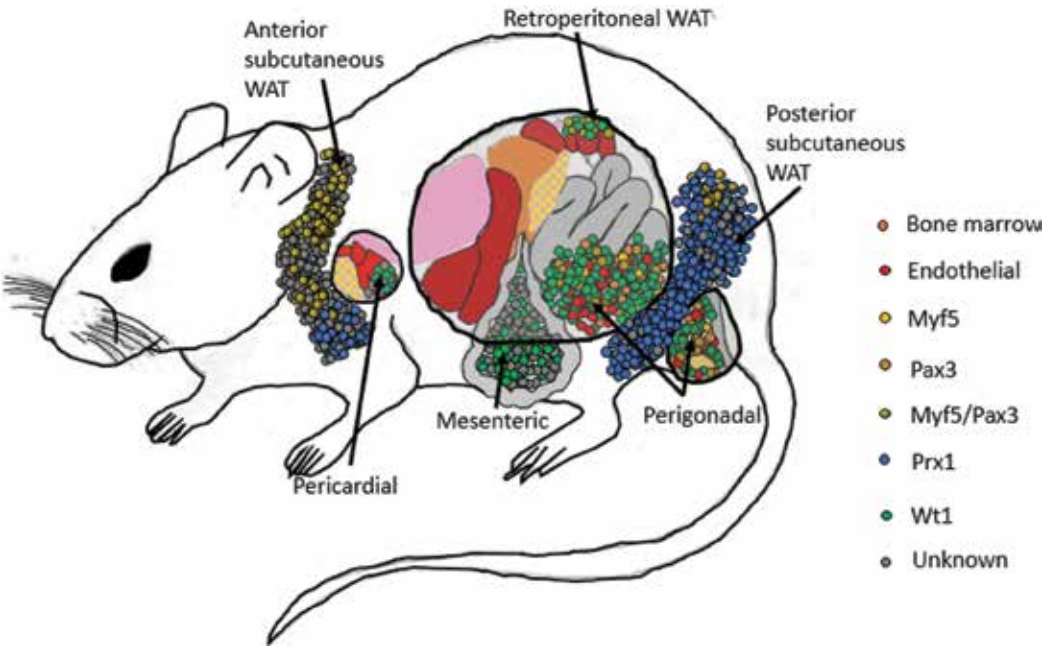

Figure 2. Intra-depot heterogeneity of WAT in mice. Bone marrow-derived adipocytes makes up about 5–10% perigonadal depot without induction of rosiglitazone or high-fat diet. Endothelial cell-derived adipocytes were also found in perigonadal depot. Myf5 and Pax3 share overlapping distribution, mostly in the anterior subcutaneous and retroperitoneal WAT. Prx1-derived pre-adipocytes were mostly found in the posterior subcutaneous WAT (75%), with a small degree in the anterior subcutaneous WAT (15%). Wt1-derived pre-adipocytes were present in only visceral depots including perigonadal (77%), pericardial (66%), retroperitoneum (50%), and mesenteric WAT (28%).

pro-inflammatory factor Pknox1, and leads to switch to M2 macrophages [224, 225]. Taken together, these studies and others demonstrate that miRNAs controls adipose tissue biology and obesity-associated pathologies through autocrine, paracrine, and endocrine actions.

7. Intra-depot heterogeneity of white adipose tissue

In addition to the differences between visceral and subcutaneous adipose tissue, growing evidence suggest that adipocytes, even within a single fat pad, are heterogeneous in nature. This heterogeneity is observable in metabolic measurements of adipocytes. These studies found that glucose uptake, lipogenesis, lipolytic response, lipid accumulation, glycolysis vs. oxidative phosphorylation, and uptake of fatty acids were markedly heterogeneous even within size-matched adipocytes of a single fat depot [226–230]. Similarly, heterogeneity in the lipolytic response of human omental adipocytes to catecholamines was previously described. These differences were at least in part, attributed to the expression of different adrenergic receptors [231]. Furthermore, ablation of hormone-sensitive lipase (HSL) or fat specific ablation of the insulin receptor lead to a polarization of adipocytes into large and small cells, thus unmasking an intrinsic heterogeneity [232, 233].

Lineage tracing analysis has been instrumental in elucidating both inter- and intra-depot heterogeneity, and the developmental origins of adipocyte lineages. In both chicken embryos and mouse embryos, populations of adipocytes in the head and thoracic regions are developmentally derived from neural crest cells [234, 235]. Although some reports suggest that adipocytes can be derived from an endothelial cell lineage both *in vitro* and i*n vivo* [236, 237], other reports dispute this claim [238]. Furthermore, studies indicate that a subset of visceral adipocytes are derived from a hematopoietic origin [239–241]. Another subpopulation of visceral adipocytes are derived from the mesothelial cells [242]. Finally, the myogenic lineage, once thought to only give rise to muscle and brown fat, gives rise to a subpopulation of white adipose tissue as well. This lineage, marked by the expression of myogenic factor 5 (Myf5) and paired box gene 3 (Pax3) give rise to adipocytes predominantly in the dorsal-anterior region, including adipocytes from the anterior subcutaneous and retroperitoneal visceral depot. This adipocyte subpopulation is dynamically distributed and its contribution to fat depots is altered in response to high fat diet and age [243] (**Figure 2**).

8. Conclusions

In summary, WAT is highly heterogeneous endocrine organ. The compartmentalization of adipose tissue into separate depots within the body is due to different developmental origins of the precursor cells. In addition, even within adipose tissue depots, individual adipocytes display developmental, genetic, and functional differences. The inter- and intra-depot heterogeneity of both preadipocytes and mature adipocytes have profound effects on whole-body metabolism, due to cell-autonomous differences in glucose and fatty acid metabolism. This heterogeneity also results in the differential inflammatory response between WAT depots. Furthermore, the differential expression of inflammatory cytokines, adipokines, and novel signaling molecules including lipokines and miRNAs between adipose depots impact the action not only of adipose tissue, but of other target tissues as well. Almost all of these factors are influenced by obesity, diet, gender, and age. Further studies to refine current knowledge on the heterogeneity of WAT may provide unique ways to manipulate physiology and lead new targets in the treatment of obesity and related disorders.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Chapter 10

Adipose Tissue as an Endocrine Organ

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Additional information is available at the end of the chapter

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Abstract

As one of the largest endocrine organs in the body, adipose tissue secrets a number of bioactive hormones, called adipokines. The expression and secretion of adipokines are tightly controlled and coordinated by physiological and pathophysiological conditions. In multiple physiological conditions, such as obesity, cold adaptation, exercise training, expression and secretion of adipokines are altered accordingly, which in turn modulate the metabolism of the whole body in endocrine, paracrine and autocrine manners. The varied changes in adipose tissues are pivotal mediators that aid the body to adapt to various physiological and pathological conditions, whereas almost all obesity-associated diseases are attributable to dysregulation of adipokines.

Keywords: adipokines, obesity, cold adaptation, exercise, inflammation

1. Introduction

For the past two decades, a lot of our knowledge on adipose tissues have been revolutionized by a series of convincing studies using animal models and cell models [1]. In contrast to the classical view that adipose tissue serves as an inert organ for storing excess energy as fat, it is now well acknowledged that adipose tissues are actually one of the largest and most dynamic organs in our body and play pivotal roles in regulation of energy homeostasis [2]. In response to alterations in nutritional status, such as excess calorie intake, fasting, lower temperature exposure, exercise, adipose tissues are among the first organs to respond. In addition to lipolysis and fatty acid synthesis, they also undergo extensive remodeling in various aspects, including in cell size and morphology, angiogenesis, normoxia/hypoxia responses, whitening/browning features, immune reactions. Most importantly, reprogramming of various pathways within adipose tissues eventually leads to substantial changes in

the expression/secretion patterns of adipose tissues, which expands and perpetuates the local influences systemically.

2. The secretory character of the adipose tissue

The adipose tissue used to be considered a simple organ where lipid is stored. Its main function was thought to be mainly mechanical in that adipose tissue was believed to serve as a cushion in our body to help protect the inner organ from bumping and damaging. In addition, people also believe that the subcutaneous adipose tissue is the insulator that prevents the heat loss of the body. The first groundbreaking discovery indicating that adipose tissue is something beyond cushion and insulator is the work by Dr. GS Hotamisligil and BM Spiegelman, who revealed for the first time that an induction of tumor necrosis factor-alpha (TNF-alpha) messenger RNA was observed in adipose tissue from four different rodent models of obesity and diabetes [3]. In accompany, protein level of TNF-alpha was also found elevated locally and systemically and neutralization of TNF-alpha significantly alleviated glucose intolerance in obese rat models [3]. This study was among the earliest to indicate the potential secretory function of the adipose tissue. But the first direct evidence that led to the realization that adipose tissue is an unneglectable endocrine organ came from the cloning of leptin in 1994 by Friedman and his colleagues [4]. They found that leptin is secreted by fat cells into the bloodstream and acts on the brain to regulate food intake and energy expenditure [5]. Before that, the identity of leptin had been remained mysterious for over four decades with the only knowledge that there existed a circulatory factor in ob/ob mice that causes obesity. Indeed, studies afterwards revealed that leptin is in practice a pleiotropic hormone and its functions extend far wider than appetite and energy balance to encompass a multiplicity of actions, including acting as a signal molecule in reproduction and immunity [6, 7].

In addition to leptin, it is now well recognized that adipose tissue, including the adipocytes and the immune cells within it, are secreting a diverse range of protein factors and signals termed 'adipokines'. These adipokines are involved in overall metabolic regulation and considered key players in maintaining both normal functioning of the body and in pathology of a series of diseases [8].

3. The adipokines affected during onset of obesity

Obesity is now a global epidemic. It is characterized by excessive fat accumulation as a result of a chronic imbalance between energy intake and energy expenditure. In addition as a cosmetic problem, obesity per se poses a substantial health risk to several common diseases including type 2 diabetes, cardiovascular diseases, stroke, arthritis and certain types of cancer [1]. Prospective studies in a cohort of U.S. men found that absolute weight gain throughout adulthood, and waist circumference were good predictors of diabetes [9] and similar conclusion was also obtained in women soon afterwards [10]. Although multiple theories have been

proposed as the underlying mechanisms for obesity-induced metabolic abnormalities, it is generally agreed that dysfunction in the over-expanded adipose tissue, and the dysregulation in adipokines, are key molecular basis for obesity-evoked metabolic pathologies [8].

A number of adipokines have been found altered upon overweight and obesity. In particular, those pro-inflammatory ones are upregulated, including TNFα, resistin, adipocyte fatty acid binding protein (A-FABP), retinol-binding protein 4, monocyte chemoattractant protein 1 (MCP1), interleukin 6 and etc. [11], whereas those with favorable functions are downregulated, such as adiponectin [12].

3.1. A-FABP—more than just a lipid chaperon

Fatty acid binding proteins are intracellular lipid chaperones that constitute a group of molecules with molecular weight of 14–15 kDa [13]. They reversibly bind to hydrophobic ligands, such as saturated and unsaturated long-chain fatty acids, eicosanoids and other related compounds (bile acids or retinoids) in their characteristic internal cavity with high affinity to coordinate lipid responses in various cells [14]. As told by its name, A-FABP is most abundantly expressed in mature adipocytes [15], due to the fact that expression of A-FABP is highly regulated during adipogenic differentiation, and its mRNA is transcriptionally controlled by fatty acids, peroxisome proliferator activator receptor gamma (PPAR-γ) agonists and insulin as well [13, 16].

A-FABP is the best characterized member in FABP family due to the striking phenotype observed in A-FABP knockout mice. Mice with a null mutation in aP2, the gene encoding A-FABP, were morbidly obese [17]. However, unlike their obese wild-type counterparts, control mice, they were devoid of insulin resistance or diabetes [17]. The protection against insulin resistance was at least partially caused by suppressed inflammation within adipose tissue, since obese A-FABP knockout animals exhibited minimal expression level of TNFα in adipose tissue. The idea that A-FABP works at the crossroad of obesity and metabolic syndrome was further highlighted by the finding that mice deficient of A-FABP were less prone to develop atherosclerotic plaques in apolipoprotein E (ApoE)-deficient mice [18]. Moreover, small molecules targeting A-FABP have been proved efficacious to deter a series of diseases or physiological conditions, including atherosclerosis [19], acute liver injury and non-alcoholic fatty liver disease [20], endothelium dysfunction [21] and etc.

Although cloned in 1983, A-FABP has not been recognized as a secretory protein until one decade ago [22]. During identification of proteins secreted from adipocytes using tandem mass spectrometry-based proteomic analysis, Xu et al. found A-FABP is present at high level in culture medium from differentiated 3T3L1-adipocytes [22]. The presence of A-FABP was further verified in persons as well (121 men and 108 women; age range, 33–72 years). Further analysis demonstrated that age- and sex-adjusted serum A-FABP concentrations correlated positively ($P < 0.005$) with waist circumference, blood pressure, dyslipidemia, fasting insulin, and the homeostasis model assessment insulin resistance index [22]. Moreover, a significant increase in A-FABP concentrations corresponding with increases in the number of components of the metabolic syndrome was also observed. The same group further evaluated the

prospective association of A-FABP with the metabolic syndrome in 495 nondiabetic adults followed up for 5 years, which revealed that subjects with higher baseline A-FABP levels had progressively worse cardiometabolic risk profile and increasing risk of the MetS. A-FABP was the independent predictor of the development of the MetS even after adjustment for each of its individual components [23]. After that, the notion that A-FABP is a circulating biomarker closely associated with obesity and components of the metabolic syndrome has been implicated by a large number of epidemiological studies on different ethnic groups [24]. These studies revealed a close association between serum levels of A-FABP and a cluster of obesity-related cardiometabolic risk factors and complications. In both cross-sectional and prospective studies, plasma levels of A-FABP are positively correlated with several key components of the metabolic syndrome, including adverse lipid profiles, hyperglycemia, hypertension and nonalcoholic fatty liver disease, independently of sex, age, and adiposity [23, 25–27].

The clinical implication and mechanisms of action of A-FABP have been extensively studied. However, much is still left unknown, especially that there are emerging target cell types and organs that are reportedly to respond to A-FABP, with expanding functions. Moreover, the putative receptor of A-FABP is still unknown and thus leaves open whether A-FABP works exclusively in an intracellular manner or also acts via cell surface receptors. Future studies addressing these questions will further enhance the understanding and the therapeutic application of this adipokine.

3.2. Hypoadiponectinemia and metabolic syndromes

Adiponectin is one of the most abundant adipokines in circulation (accounting for 0.01% of total serum protein), and was cloned by three independent groups in 1996 [28–30]. Since its discovery, it has been receiving intense interests, because of its negative association with obesity, which is in sharp contrast to most of the adipokines identified [31]. The monomeric adiponectin is a 30 kDa protein with a C-terminal globular domain and a collagen-like N-terminal domain [32]. However, under physiological settings, adiponectin monomer has never been detected. Instead, its predominant forms in circulation are trimer, hexamer and oligomers (18mers or higher), which are called low-, medium- and high-molecular weight form of adiponectin, accordingly.

Circulating level of adiponectin is tightly controlled at every step, from its transcription, translation, multimer-assembly, and finally to secretion [33]. Therefore, it is not surprising to find that adiponectin is present in blood stream at varied concentrations and multimer ratios as well. Indeed, reduction of adiponectin has been consistently observed in obese adipose tissues at transcriptional levels and in circulation, in both animal models and clinical samples [34]. However, it should also be noted that compared to the total serum level of adiponectin, serum HMW adiponectin values are more relevant to the presence and severity of metabolic syndromes [35]. Multiple clinical studies have reported the inverse association between high-molecular weight (HMW) adiponectin and triglycerides, blood pressure, obesity and fasting glucose, while is positively associated with high density lipoprotein (HDL) cholesterol, the so-called `good cholesterol' [35–38].

Consistent with its reduced level in obesity and related complications, animal and cell based studies have unequivocally showed that adiponectin indeed exerts a multiplex of favorable functions against almost all the metabolic dysfunctions examined [24]. The protective functions of adiponectin are mainly attributed to its anti-diabetic, anti-inflammation, and vascular-protective functions. It is worthy to note that although adiponectin is exclusively expressed in adipocytes, the receptors for adiponectin [39, 40], the adipoR1, adipoR2 and T cadherin, are widely expressed throughout the body and thereby facilitate the pleotrophic action of this favorable adipokine. Mechanistically, it was shown that phosphorylation and activation of the 5′-AMP-activated protein kinase (AMPK) are stimulated by adiponectin in skeletal muscle and in the liver [41]. In accompany with this, phosphorylation of acetyl coenzyme A carboxylase (ACC), fatty-acid oxidation, glucose uptake and lactate production in myocytes, and phosphorylation of ACC and reduction of molecules involved in gluconeogenesis in the liver were observed, which eventually led to reduction of glucose levels in vivo [41]. Moreover, adiponectin can directly act on inflammatory cells and suppresses reactive oxygen species which in turn stimulates the expression of the anti-inflammatory cytokine IL-10, and suppresses the NF-κB inflammatory signaling pathway, and downregulates the key inflammatory cytokine TNF-α [42, 43]. Protection against cardiovascular diseases by adiponectin is achieved by its actions on various cell types in cardiovascular system, including endothelial cells, macrophages/monocytes, smooth muscle cells, cardiac muscle cells and etc. (reviewed by [34, 44]).

The exact reason in terms of why adiponectin expression is reduced in obese adipose tissue still remains elusive. However, the presence of FGF21 resistance might be a potential mechanism (discussed below).

3.3. Fibroblast growth factor 21 (FGF21) resistance in obesity

The biological function of FGF21 was firstly recognized in a high throughput screening assay for potent stimulator of glucose uptake in mouse 3T3-L1 and primary human adipocytes, during which FGF21 was identified as a positive hit [45]. Further studies revealed that in addition to its glucose-lowering effect, FGF21 is also capable of improving lipid metabolism [46], and is therefore regarded as a new molecule with therapeutic potentials. Although the detailed mechanism is not clear, FGF21 is a potent activator of extracellular signal-regulated kinase (ERK), with a 10-fold higher potency in adipose tissue than in liver, suggesting that FGF21 may preferentially target adipose tissue [47]. Indeed, two back-to-back studies reported that a majority of the biological functions of FGF21 was mediated via its inducing action on adiponectin expression [48, 49]. These studies provide feasible explanation for the previous findings that adiponectin and FGF21 share a plethora of profound benefits on cardiometabolic events, spanning from glucose lowering, lipid lowering to cardiovascular-protective properties, despite of their distinct origin of production and structure. Specifically, FGF21 and adiponectin deficient mice are both more susceptible to high fat diet induced hyperglycemia, insulin resistance, non-alcoholic fatty liver disease and a number of cardiovascular dysfunctions [50]. On the other hand, pharmacological administration of either adiponectin or FGF21 recombinant proteins or analogues was both proved to be efficacious in alleviating a series of metabolic perturbations in diseased rodent models and non-human primates [51–54].

Although a number of pharmacological studies support for the FGF21-adiponectin axis, the correlation between FGF21 and adiponectin is distorted at physiological and pathological conditions. Unlike its downstream effector adiponectin, whose circulating level is found to be reduced in overweight/obesity and metabolic syndromes [34], obesity is accompanied with elevated levels of serum FGF21 in both rodent models and humans [55]. Moreover, positive correlations has been documented between FGF21 and a panel of metabolic syndromes, including insulin resistance, type 2 diabetes, dyslipidemia, non-alcoholic fatty liver disease, coronary heart disease and polycystic ovarian syndrome [56]. Our earlier study only found a weak negative correlation between serum FGF21 and adiponectin and the significance was lost after adjustment for BMI [55]. Likewise, a recent clinical study in patients with metabolic syndrome confirmed the lack of association between FGF21 and adiponectin, although FGF21 was found strongly correlated with A-FABP, another adipokine with unfavorable functions [57].

The dissociation between adiponectin and FGF21 in obese condition can be explained by the state of FGF21 resistance. This is evidenced by the blunted response to FGF21 administration in liver and adipose tissues from obese animals [55, 58]. At the molecular level, the attenuation of FGF21 action in obesity can be attributed to the selective reduction of β-Klotho in white adipose tissue, which is commonly observed in rodent models of obesity and in obese individuals [59].

4. The adipokines affected upon cold adaptation

Adipose tissue is a highly heterogeneous organ that it can be classified as white adipose tissue (WAT) and brown adipose tissue (BAT), in terms of their function to either store excess energy or to dissipate energy. The latter function is achieved by uncoupling protein-1 (UCP1), which converts the chemical energy into heat [60, 61]. For a long time, the BAT was believed only present in infants; however, the advancement in positron emission tomography (PET) scan technology revealed considerable amount of functional BAT in adult human [62]. Interestingly, a major portion of the functional BAT in adults is "inducible,, upon cold stimulation, which seems to be elicited by both sympathetic nervous system (SNS) dependent and independent mechanisms. Though BAT has great anti-obese potential, animal studies have demonstrated that it possesses systemic metabolic benefits beyond its adiposity-reducing effect, including insulin-sensitizing, lipid-lowering, anti-inflammation and anti-atherosclerosis [63, 64]. Thereafter, it is conceivable that cold-induced adipose browning confers favorable effects at least partially via modulation of its secretory pattern.

4.1. The proinflammatory adipokines in response to cold

Ten lean, healthy male volunteers were exposed to cold for 2 h and compared with a control group of 10 subjects without exposure to cold [65]. It is found that the plasma concentration of MCP1 was increased by cold temperature while no specific changes were observed for IL-6 and VEGF [65]. The observed alterations appeared to be posttranscriptional because adipokine gene expression was found to be unaltered. Another study in mice also examine

the temporal profile of cold-induced changes in the expression of inflammatory adipokines in adipose tissues or primary adipocytes [66]. To this end, male C57BL/6J mice were exposed to 4°C for 1–5 days. Gene and protein profiling revealed that the inflammatory adipokines were expressed significantly higher in WAT than BAT at baseline [66]. Moreover, it was shown that the browning process alters the inflammatory adipokines expression in adipose tissues, which is dynamically decreased in sWAT whilst increased in eWAT for compensation [66]. In our study, we also found a transient increase of pro-inflammatory cytokines, such as TNFα, MCP-1, in white adipose tissue of mice when they were put to cold environment, especially in subcutaneous adipose tissue [67]. However, longer term cold adaptation led to a remarkable decrease of these proinflammatory cytokines [67], which coincided with the sharp increase of those anti-inflammatory cytokines.

4.2. Type 2 immune cytokines in cold-stimulated white adipose tissue

It is now known that the classical BAT present in new born baby and those inducible ones scattered in white adipose tissue are fundamentally different, although they are both positive for the brown adipocyte marker UCP-1 [1]. One of the major differences is reliance on type 2 immunity for activation or not [68]. Qiu et al. reported that cold exposure is accompanied by elevation of the Th2 cytokines (mainly interleukin 4 from eosinophils), which can readily engage M2 macrophage activation [68]. The latter one serves as the primary local source of catecholamine for biogenesis of brown-like adipocytes [69]. Later studies found that upon cold challenge, meteorin-like (Metrnl) was induced in adipose tissue [70]. More importantly, Metrnl stimulates an increase in IL-4 expression in an eosinophil-dependent manner and thereby promotes alternative activation of adipose tissue macrophages. Conversely, blockade of Metrnl actions in vivo significantly attenuates chronic cold-exposure-induced alternative macrophage activation and thermogenic reprogramming [70]. Although other studies also suggest that ILC2 cell-derived factors, including interleukin 5 and interleukin 13, act upstream of eosinophil-M2 macrophage cascade during cold-induced adipose browning [71, 72], either the number of ILC2, or its stimulating factor IL33, were not found to be significantly altered by cold stimulation, though they are remarkably decreased in obese conditions [71, 72]. This suggests that there exist other physiological cues that entitle the type 2 immune response during cold adaptation.

4.3. FGF21-adiponectin axis in cold adaptation

FGF21 has long been known as a pro-browning factor for classical brown adipocytes and was found induced in BAT after cold exposure [73, 74]. Recently it is found that FGF21 expression was also strongly upregulated in white adipocytes under cold-acclimated conditions [75]. By using the adipocyte-specific FGF21 knockout mice, it was further revealed that adipocyte-derived FGF21 acts in an autocrine manner to promote the expression and secretion of CCL11 via activation of ERK1/2 [75]. This event then drives recruitment of eosinophils into subcutaneous WAT, leading to increases in accumulation of M2 macrophages, and proliferation/commitment of adipocyte precursors into beige adipocytes. Neutralization of CCL11 abolished all these events induced by FGF21, and thus demonstrating that the adipose-derived FGF21-CCL11

cascade triggers cold-induced browning and thermogenesis by coupling sympathetic nervous system to activation of type 2 immunity.

On the other hand, considering that adiponectin is induced by and acts downstream of FGF21 at certain conditions as previously reported [48, 49], whether the effect of FGF21 to enhance white adipose browning is partially mediated by adiponectin. However, despite of the fact that transcription of adiponectin was remarkably induced within white adipose tissue upon cold challenge [67], such a change was not abolished adipose-specific deletion of FGF21 or its co-receptor β Klotho [75], indicating that cold-evoked upregulation of adiponectin in adipose tissue was not controlled under FGF21. Moreover, although adiponectin deficient mice were also less sensitive to white adipose browning stimulated by cold [67, 76], it seems that it activates browning remodeling in distinct ways as FGF21. It may on one hand acts on M2 macrophages by promoting their de novo proliferation; while on the other hand promotes mitochondrial biogenesis, insulin signaling, and the AMPK-SIRT1 pathway and up-regulates β3-adrenergic receptor in BAT [76]. However, it should be notified that opposite findings were also reported [77] and whether the inconsistencies were caused by the different strains of mice used, or gut microbiota in different housing environment, needs to be investigated in future.

5. The adipokines changed by exercise

Physical activities are one of the major ways of energy expenditure. Sufficient epidemiological evidences have shown that there exists an inverse association between physical activity and obesity [78]. Exercise has also been demonstrated as an effective non-pharmaceutical therapeutic method to mitigate obesity and obesity-related metabolic syndromes such as type 2 diabetes and cardiovascular diseases [79, 80]. In addition to directly increasing the energy expenditure, exercise also modulates the functions of many organs including muscle, liver, heart and adipose tissue through different pathways to improve the systemic metabolisms [81, 82]. The cross-talk between the muscle and the adipose tissue is also involved in this process and the adipokines secreted from the adipose tissue might play important roles in the effects of exercise.

5.1. Adiponectin in exercise

Plenty of clinical study focused on the relationship between exercise and the plasma (or serum) adiponectin levels and the results are largely dependent on the duration and intensity of the exercise [83–85].

In most studies, one single exercise could not show acute influence on the plasma adiponectin level, no matter the duration of exercise or whether the subjects are healthy or obese [84, 86–89]. However, an article published in 2010 reported that both high and low intensity anaerobic exercise could elevate blood adiponectin level in obese men [90], which is similar to the result of another report in 2006 using healthy men with anaerobic exercise [91]. In addition,

not only the total concentration but also the profile (percentage of HMW and LMW/MMW) of adiponectin was reported to be changed by exercise, which shows that the activity and the functions of adiponectin may also be influenced by the exercise [92].

About the choric effects of training on adiponectin, the results with different training protocol are also conflicting [84]. Kriketos et al. reported in 2004 that with aerobic exercise, the adiponectin level of obese subject will rise from 7.0 ± 0.7 to 18.2 ± 1.9 μg/ml, after about 1 week and remained throughout the 10 weeks of training [93]. This result is similar to Kondo et al.'s work in which they reported that after 7 months training, the plasma level of obese group increased obviously but the control group remains unchanged [94]. However, in some other experiments which were also performed on obese subjects with 2–3 months training, the adiponectin level was not significantly changed [94, 95]. Two systematic review which analyzed the results of numerous experiments both got the conclusion that adiponectin level could be upregulated by the exercise [85, 96]. And, interestingly, Khoo et al. compared dieting and exercise, the two common obesity therapies and found that after 24 weeks of treatment, both methods could effectively lead to weight loss, but adiponectin is upregulated only in the exercise group but not the dieting group [97].

The inconsistence of the results might be caused by the inconsistent factors in these experiments including the type and detailed protocols of exercise (aerobic or anaerobic, duration, intensity, etc.), subjects (gender, race, age, obese or lean, etc.) and diet condition (with or without controlled food intake). This shows that adiponectin level in blood is sensitive to these confounding factors. However, at least in part of the experiments, the beneficial effects of exercise are partially mediated by adiponectin.

5.2. Alteration of leptin by exercise training

Similar to adiponectin, the effects of exercise also depend on the duration and intensity of the exercise. Considering that the expression and release of the leptin by the adipose tissue is closely related to the energy homeostasis and low energy state will decrease the leptin level [98], the leptin level shows inverse association with the energy expenditure after a single bout of exercise. In a study which the author compared the leptin level after three kinds of exercise and found that leptin level decreased obviously after ultramarathon race (with about 7000 kcal energy expenditure) and ski-alpinism race (with about 5000 kcal energy expenditure) but not half-marathon run (with about 1400 kcal energy expenditure) [99]. Kraemer et al. summarized the works done by different groups and concluded that >60 min exercise is more likely to induce the decreasing of leptin compared with short-term exercise [100]. Some other groups reported that high intensity of short term (20–30 min) exercise can also down-regulate the blood leptin level [101, 102].

Another study reported that after the first time of 45-min walking, there were no obvious changes in the plasma leptin level. However, after chronic training (>7 times of the same walking exercise), the leptin decreased obviously, which shows that chronic training may be more effective to the decrease of leptin [103]. Bouassida et al. analyzed the 10 previous papers in which the chronic effects of exercise to leptin were studied and found that in all the trainings

that are longer than 3 weeks, the decreasing of leptin was observed no matter the subjects are obese or healthy [85].

Considering the functions of leptin talked in previous parts, the decreasing level of leptin will lead to the increase of appetite and food intake and reduce the energy expenditure, which is the compensatory effects of the weight loss in exercise.

5.3. Inflammatory adipokines influenced by exercise

Obesity induced adipose tissue inflammation is closely related to the insulin resistance and adipose tissue dysfunctions. Inflammatory adipokines, such as TNF-α, IL-6 and chemerin, play crucial roles in the chronic inflammation and the recruitment and activation of immune cells.

Nicklas et al. reviewed the previous research and found that there is inverse association between the level of systemic inflammation markers, including TNF-α and IL-6, with the physical activity, which provide epidemiological evidence to the effects of exercise on inflammatory adipokines [104]. For the patients with obesity or diabetes, plenty of studies showed that the long-term training can decrease the level of TNF-α or/and IL-6 [83, 105–108], which demonstrate that the beneficial effects of exercise is partly mediated by decreasing the systemic inflammation.

In addition, in the experiments of Khoo et al., it was found that the long term exercise effective down specifically regulated the serum high-sensitivity C-reactive protein (CRP), which is a an down-stream factors of IL-6, in obese group but not in health group [97]. This indicates that effects of long term exercise might be different for obese subjects and lean subjects, considering their basal levels of adipokines before exercise are also different [109].

In addition to these adipokines that have been repeatedly measured in various experiments, there are increasing number of papers focusing on the relationship of other adipokines and exercise. For example, apelin was found to be upregulated by exercise. Study of the relationship between exercise and the adipokines and the functions of these adipokines may contribute to the understanding of the underlying mechanisms of the beneficial effects of exercise and provides new strategies for developing anti-obesity therapies and exercise mimicking drugs.

6. Conclusions

Adipose tissue is gaining increasing interest in the past decade, in both basic research and pharmaceutical industry, owing to the realization that it serves as a commander of the whole-body metabolism. Conceivably, the regulatory function of adipose tissue is almost exclusively mediated by the adipokines, along with other small bioactive molecules (which is not discussed here). Although much progress has been made to decipher the underlying mechanism whereby adipose-derived factors contribute to physiological and pathological conditions, a lot more still remain unanswered. In particular, a number of inconsistencies exist in expression

and mechanisms of the adipokines which hinder the elucidation of their biological functions. However, on the other hand, it is also encouraging that several adipokine-based pharmacological agents have been developed, such as adiponectin receptor agonist [110], PEGylated FGF-21 [111], and neutralizing antibody against IL-1β [112], all of which have shown promising effects against metabolic-related diseases. A better understanding on adipokine biology will further benefit the design and development of novel classes of therapeutics with less side-effects.

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Conflict of interest

The authors claim no conflict of interest.

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Section 5

Signaling System in Adipose Tissue

Chapter 11

Feedback Control of Second Messengers Signaling Systems in White Adipose Tissue Adipocytes in Healthy State and Its Loss at Adiposity

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Additional information is available at the end of the chapter

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Abstract

Second messengers Ca^{2+}, IP3, cAMP, NO, cGMP, and cADP ribose are incorporated as obligatory elements into multivariable Ca^{2+}-signaling system, which integrates incoming signals of hormones and neurotransmitters in white adipocytes. This cross-controlled system includes two robust generators (RGs) of rhythmic processes, involving phospholipase C- and NO-synthase-dependent signaling networks (PLC-RG and NOS-RG). Multi-loop positive feedback control of both RGs provides their robustness, multistability, signaling interplay, and extreme sensitivity to the alterations of incoming signals of acetylcholine, norepinephrine, insulin, cholecystokinin, atrial natriuretic peptide, bradykinin, and so on. Hypertrophy of cultured adipocytes and of mature cells, isolated from epididymal white adipose tissue (eWAT), results in the loss of rhythmicity and development of general hormonal signaling resistance. Preadipocytes isolated from eWAT of obese mice cannot grow and accumulate lipids in the media devoid of fatty acids. However, even low concentrations of palmitoylcarnitine in the media (1 μM) may result in drastic suppression of mRNA expressions of the proteins of Ca^{2+}-signaling system, especially of NOS-RG. Similar alterations of gene expression are observed in eWAT and liver at adiposity. All this may indicate on universal background pathogenic mechanisms. Treatment modalities, which may help to restore deregulation of Ca^{2+}-signaling system and corresponding tissues dysfunction, are discussed briefly.

Keywords: adiposity, adipocyte dysfunction, second messengers, NO, PKG, feedback and cross-control, loss of rhythmicity

1. Introduction

Adipose tissue dysfunction ("adiposopathy") is considered as one of the primary drivers of multifactorial pathological process, ranging from systemic insulin resistance and hypertension to cardiovascular diseases, liver and pancreas dysfunction, and type 2 diabetes (T2D) [1, 2]. Obviously, observed gradual dysfunction of various tissues at T2D is due to the deregulation of metabolic and signaling systems providing the fulfillment of these functions. All these processes of deregulation, especially of signaling systems, may have some universal features, which are being based on the loss of feedback control mechanisms in the systems studied. The identification of these control mechanisms, including crosstalk of signaling pathways, may create new opportunities to identify real targets and develop new options of various diseases treatment.

"Adiposopathy" is characterized by: lipid metabolism deregulation, development of oxidative stress and mitochondrial dysfunction, death of hypertrophied adipocytes, tissue remodeling, loss of fatty acids buffering, and endocrine and immune functions [2–6]. However, the existing data on external hormonal and autonomous feedback control of white adipose tissue (WAT) lipid metabolism (triglycerides—fatty acids turnover) are insufficient to answer the question on the mechanisms providing uncontrolled hypertrophy of adipocytes. Later, based on own results obtained in animal experiments and known literature data, we will try to represent (at first level of approximation) the structures and mechanisms of autoregulation of second messengers signaling systems, which might be functioning in the adipocytes and other types of nonexcitable cells.

2. Calcium, cAMP, and cGMP-related signaling systems, operating in adipocytes of healthy animals

2.1. Brief survey of existing models of adipocyte triglyceride metabolism control

It is known that, acting via lipid kinase (PI3K)/PKB-signaling pathway, insulin may stimulate adipogenesis and triglyceride (TAG) synthesis, by phosphorylating rate-limiting enzymes Acyl-CoA: glycerol-3-phosphate acyltransferases (GPAT1, 4) and phosphatidic acid phosphatase (Lipin) [7, 8]. On the contrary, norepinephrine (NE) promotes dephosphorylation of lipin [8]. Modern viewpoints on the control of TAG hydrolysis to free fatty acids (FFA) are mainly focused on the regulation (phosphorylation) of hormone sensitive lipase (HSL), adipocyte triglyceride lipase (ATGL), and perilipin by PKA and are being based on opposite influence of NE (β-adrenoreceptors; β-*AR*) and of insulin on cAMP concentration and PKA activity [9–14]. Supposed mechanisms of antilipolytic action of insulin include the activation of cAMP phosphodiesterases PDE3,4 and inhibition of PKA activity through Insulin/PI3K/PKB-pathway. In addition to insulin, antilipolytic action may be provided through G-protein-coupled receptors by NE (α2-*AR*), adenosine, prostaglandins, neuropeptide Y, and so on [11–14].

Phosphorylation of key lipases and perilipin by PKG1 is considered as a separate mechanism, involved in the activation of TAG hydrolysis [10, 13, 14]. This signaling pathway, which is

based on the activation of PKG1 via atrial natriuretic peptide receptor A (NPR-A/mGC/cGMP/PKG1-pathway), does not involve nitric oxide (NO) and soluble guanylate cyclase (sGC).

This widely admitted model of TAG-FFA turnover (futile cycle) control describes the regulation of lipid metabolism as external hormonal adjustment, realized through PI3K/PKB, cAMP/PKA, and cGMP/PKG1, that is, as a model devoid of self-control and crosstalk of functioning second messenger signaling systems. Moreover, NO and calcium are not included into consideration as possible messengers, involved in the control of WAT metabolism. Though the results of the last decade indicate that the activation of endothelial NO-synthase (eNOS), NO bioavailability, and recruitment of eNOS/NO/sGC/cGMP/PKG1-signaling chain may protect against obesity, by influencing differentiation and mitochondrial biogenesis in brown fat cells, adipogenesis and lipolysis in white cells, and so on. [15–20]. Besides that, controversial results on the role of Ca^{2+} and calcium-sensing receptors in the regulation of body fat depots [21–23] point on the important role of Ca^{2+} in the mechanisms of self-control of second messengers signaling systems.

2.2. Two Ca^{2+}-dependent signaling systems and rhythmic processes in adipocytes of WAT

Like most of other nonexcitable types of cells, adipocytes possess two types of intracellular Ca^{2+} release channels, located on the membrane of endoplasmatic reticulum: inositpl-1,4,5-triphophate (IP3) receptors (IP3R) and ryanodine receptors (RyR). Both types of receptors are controlled by numerous signaling molecules, including PKA PKG, Ca^{2+}-dependent kinases, various isoforms of PKC, and so on [24–26]. This versatility of control defines the shaping of intracellular Ca^{2+} dynamics, which plays a primary role in the regulation of numerous cellular processes [24]. Ubiquitous oscillations of intracellular Ca^{2+} concentration, which are observed in most of nonexcitable cells [27–30], are often considered as the basic dynamic mechanisms involved in the control of cellular metabolic processes [27–29]. However, the role of Ca^{2+} oscillations and of triggering phenomena is not understood and evaluated yet properly [27, 30]. However, the analysis of such dynamic processes may be very instrumental for the determination and evaluation of operating feedback mechanisms.

Both types of Ca^{2+} release channels possess a fundamental property, called Ca^{2+}-induced Ca^{2+} release (CICR), which may provide Ca^{2+}-sparks, fast oscillations, and spatial waves [24–26]. Gaiting of IP3R is reinforced by IP3, which facilitates binding of Ca^{2+} and channel opening [24, 25]. In other words, Ca^{2+} and IP3 represent crosscoupled messengers targeted to IP3R [31].

As for RyR, according to generally accepted point of view, the regulation of this receptor lacks this kind of symmetry. Cyclic ADP-ribose (cADPr), which is formed from NAD by ADP-ribosyl cyclase (ARC) or ectoenzyme CD38, is not considered as an obligatory coagonist of RyR [26], in spite of existing data on its modulatory role in RyR-channels gaiting and CIRC control [32–37]. Really, in striated muscles RyR-channels gaiting and CIRC are determined mainly by plasmalemmal membrane depolarization [26]. In nonexcitable cells, the primary role in Ca^{2+} homeostasis is supposed to be realized via IP3R [24, 27–30], while modulatory role is delegated to RyR, which may amplify Ca^{2+}-signals produced by IP3-dependent CICR [24–26].

2.2.1. Ca^{2+}/phospholipase C/IP3/IP3R/Ca^{2+} positive feedback signaling system

Numerous external signals, by stimulating Gq proteins and tyrosine kinase (TK) coupled receptors, result in the formation of IP3 by various isoforms of phospholipase C (PLC) [24, 25, 38] with subsequent activation of IP3R-channels and rise of Ca^{2+}in the cytoplasm via CICR mechanism:

$$\mathrm{TK}, G_{\alpha q} \rightarrow \mathrm{PLC} \rightarrow IP_3 \rightarrow IP_3R \rightarrow Ca^{2+} \quad (1)$$

Realization of IP3-dependent CICR represents short positive feedback loop (PFL) in the system:

$$Ca^{2+} \rightarrow IP_3R \rightarrow Ca^{2+} \quad (2)$$

Being activated by Ca^{2+}, Ca^{2+}-dependent isoforms of PLC may provide functioning of long PFLs [31]:

$$Ca^{2+} \rightarrow \mathrm{PLC} \rightarrow IP_3 \rightarrow IP_3R \rightarrow Ca^{2+} \quad (3)$$

Therefore, IP3R-dependent Ca^{2+}–signaling system represents two loops' generator (**Figure 1**), in which short PFL (shown as broken arrow 1) is embedded by long PFL (arrow 2). This duplicating loop may provide the robustness with respect to the alteration of systems parameters [39, 40]. Released by IP3R-channel intracellular Ca^{2+} may provoke RyR-dependent CICR, which, in turn, might further amplify initial signals and support generation of Ca^{2+} oscillations and/or wave propagation. Inhibition of IP3R, due to phosphorylation of IP3R by Ca^{2+}-activated CaM-kinases II (CaMKII), represents stabilizing negative feedback loop (NFL) (dotted line 4 with sign T).

This Ca^{2+}/PLC/IP3/IP3R/Ca^{2+}-robust generator (PLC-RG) is cross-activated by adenylate cyclase (AC)/cAMP/PKA-signaling pathway, owing to phosphorylating of IP3R (and RyR) by PKA (arrows 7, 8). Inhibition of AC and activation of PDE3, produced by the phosphorylation of both enzymes by PKA [42], may provide the functioning of two stabilizing NFLs in this pathway (dotted line 5 and arrow 6). And finally, PI3K/PKB/NO/cGMP/PKG1-signaling pathway participates in negative crosstalk with both systems, by inhibiting IP3R via PKB (dotted line 10) and PKG1 (dotted line 12) and by activating PDE3 through PKB (arrow 9) [10, 11]. Well-known inhibition of PDE3B by cGMP (dotted line 11) [9] contradicts this logic of system's self-control and, apparently, may be realized at high concentrations of cGMP.

Cross-inhibition of eNOS, based on its phosphorylation by PKC [41], is shown at the bottom of **Figure 1** (dotted line 14). Cross-inhibition of PLCβ activity, which may be realized with the involvement of PKG1 and PKA [42], is omitted for the simplicity.

It ought to outline that, besides combined action of IP3 and Ca^{2+} on IP3R, PLC-RG has second point of regulatory symmetry. The entry into the system through PLC is carried out by combined activation of PLC by $G_{\alpha q}$-proteins (or TKs) and Ca^{2+} (**Figure 1**).

PLC-RG represents a highly nonlinear dynamic system, which incorporates the family of two nested PFLs. Due to that, this system possesses the properties of multistable generator, which

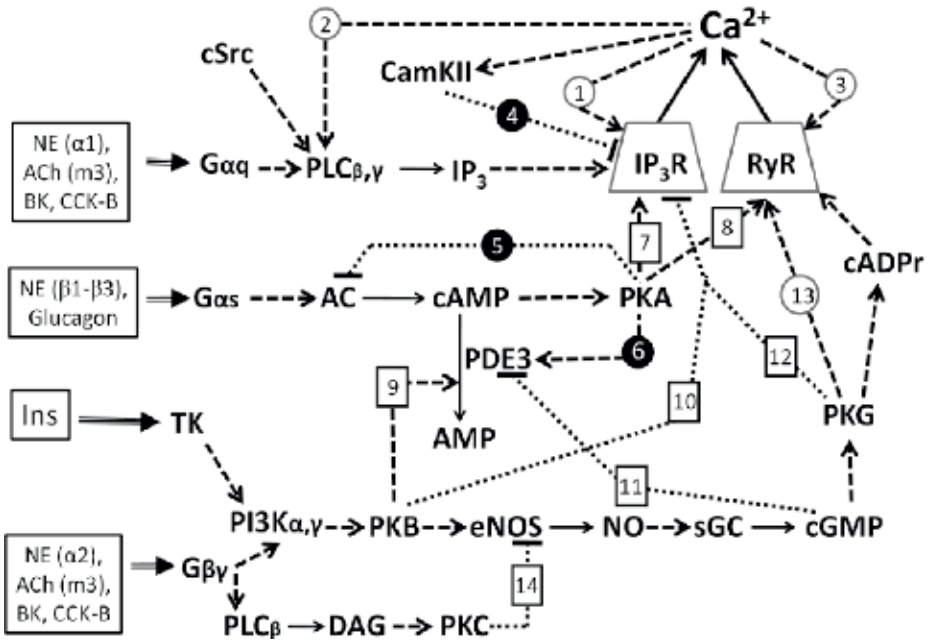

Figure 1. PLC/IP3/IP3R/Ca^{2+}-signaling system and its cross-control by AC/cAMP/PKA and PI3K/PKB/NO/cGMP/PKG-signaling pathways. All abbreviations and explanations are given in the text. Various types of activation and inhibition (direct regulations or covalent modifications) are indicated as broken arrows and dotted lines (with symbol T), correspondingly. Positive and negative feedback loops are marked with white or black circles and have the numbers 1–3 and 4–6, correspondingly. Crosstalk loops are marked by the squares and have the numbers 8–12 and 14. Various hormones and neurotransmitters (with corresponding receptors), activating G-proteins and TKs, are placed in the boxes.

may produce: steady state regimes with different concentrations of intracellular Ca^{2+} (and of other second messengers), triggering phenomena, Ca^{2+} spikes, ordinary and complex (multiperiodic or chaotic) oscillations, and waves propagation. Realization of described regimes depends on the system's parameter values, that is, on the set of enzyme and channel activities and agonist affinities. All such regimes were observed experimentally in various types of cells [43–51] and were reproduced in mathematical models [52–54]. Apparently, all ranges of these dynamic regimes were observed for the first time on isolated hepatocytes [45]. In most of the published experiments registered Ca^{2+} oscillations and waves were attributed to the functioning of PLC-RG, including regimes elicited by NE [45], Ach [47, 49], histamine [44], glutamate [48], and so on.

In cultured adipocytes of epididymal WAT, periodic Ca^{2+} signals and spikes, which depend on PLC-RG activity, may be evoked by fetal bovine serum [50, 51], NE [55–57], Angiotensin II (Ang II) [58, 60], cholecystokinin (CCK) and ANP [58], insulin [59], and bradykinin (BK) (Turovsky et al., submitted for publication).

2.2.2. Ca^{2+}/eNOS/NO/sGC/cGMP/PKG/ARC/cADPr/RyR/ Ca^{2+} positive feedback signaling system

In contrast to all abovementioned agonists, ACh may elicit Ca^{2+} oscillations in WAT adipocytes (9DIV) by involving another mechanism, which does not implicate PLC and IP3R [61].

The effect of ACh is realized via *m3*-muscarinic receptors (*m3-MR*) and Gβγ subunits of corresponding Gq-proteins. This kind of rhythmic activity is characterized by Ca^{2+} and NO oscillations with phase shift about 180°. Remarkably, insulin, Ang II, CCK, and BK may also evoke Ca^{2+} and NO oscillations by activating second oscillatory mechanism [59, 60].

Earlier works, performed by several groups, depicted that NO, cGMP, and cADPr may induce Ca^{2+} mobilization and oscillations in hepatocytes [64, 65], smooth muscle cells [66, 67], and T-cells [68], involving RyR. The model, proposed for first time to explain mobilization of intracellular Ca^{2+} via NO/cADPr-dependent signaling pathway [33, 34], was based on known phosphorylation and activation of ARC by PKG1 [36, 37] and on the facilitation of RyR-channels gaiting by newly formed cADPr [32, 33]. This model include following signaling chain:

$$\text{eNOS} \rightarrow \text{NO} \rightarrow \text{sGC} \rightarrow \text{cGMP} \rightarrow \text{PKG1} \rightarrow \text{ARC} \rightarrow \text{cADPr} \rightarrow \text{RyR} \rightarrow Ca^{2+} \quad (4)$$

Well-known activation of eNOS by Ca^{2+} [69–71] transforms this linear chain into long PFL, which creates basic loop of NOS-dependent robust generator (NOS-RG):

$$\begin{aligned} Ca^{2+} &\rightarrow \text{eNOS} \rightarrow \text{NO} \rightarrow \text{sGC} \rightarrow \text{cGMP} \\ &\rightarrow \text{PKG1} \rightarrow \text{ARC} \rightarrow \text{cADPR} \rightarrow \text{RyR} \rightarrow Ca^{2+} \end{aligned} \quad (5)$$

Speaking on the math language, formation of long PFL(5) represents necessary conditions for the functioning of NOS-RG. PFL(5) is very sensitive to any input in it. The application of ANP (input of cGMP), 8-br-cGMP, SNAP (influx of NO), NAD (substrate in the synthesis of cADPr) [61], or activation of Ca^{2+}-influx (by low concentrations of arachidonic acids via store-independent Orai channels) [63], may bring oscillations and triggering regimes in adipocytes.

ACh and all abovementioned hormones, activating TK or/and G-protein-coupled receptors, provide sufficient conditions for stable functioning of NOS-RG, activating eNOS via axis:

$$\text{TK}, G\beta\gamma \rightarrow \text{PI3K}\gamma \rightarrow \text{PKB} \rightarrow \text{eNOS} \quad (6)$$

Incubation of cultured adipocytes with the inhibitors of the proteins of this axis prevents the activation of NOS-RG by ACh, insulin, CCK, and Ang II [61–63].

Thereby, NOS-RG also has both kinds of symmetries, including: (a) activation of RyR by cross-coupled second messengers Ca^{2+} and cADPr and (b) combined activation of eNOS by Ca^{2+} and PKB (via axis (6)).

The model of self-control of NOS-RG is presented in **Figure 2**. Besides short and long PFLs, based on cADPr-dependent CICR and activation of eNOS by Ca^{2+} [69–71] (broken arrows 1, 2), this model incorporates six PFLs (arrows 3–8) and three NFLs (dotted lines 10, 12, and broken arrow 11). Group of PFLs (arrows 3–6), based on the phosphorylation and activation of eNOS [70, 71] and of PKB by CaMKIV and AMPK [72, 73], provide the amplification of basic PFLs and robustness of NOS-RG.

PKG1 occupies a central position in the autoregulation of NOS-RG. Feedforward activation of RyR [26] and feedback activation of eNOS [74–76] and PKB [72, 77] by PKG1 (arrows 7–9)

raise the reliability of this system. Feedback inhibition of sGC [78] and activation of PDEV [79] by PKG1 are directed to lower the level of cGMP in NOS-RG (line 12 and arrow 11). The inhibition of external Ca^{2+} influx, realized via inhibition of TRP channels by PKG1 [80–82] (line 10), may reinforce these NFLs.

The reliability and low sensitivity to noise and parameters alterations of technical systems is primarily attained by multiple negative feedback control and duplication of operating elements [39, 40, 83, 84]. PFLs, in contrast to NFLs, may enhance system's sensitivity to changes of internal parameters and noise by amplifying incoming signals.

Basic structures of autoregulation of PLC-RG and NOS-RG involve the families of nested PFLs. Such unusual structures may create new properties of analyzed system: combination of extreme sensitivity to the alterations of incoming signals and the reliability, provided by the redundancy of PFLs. Due to that, both systems may be considered as robust but sensitive integrators of multiple hormonal signals.

Positive cross-control of NOS-RG, fulfilled by AC/cAMP/PKA-signaling pathway, is indicated by broken arrows 13–15 in **Figure 2**. This control is directed to internal elements of main PFLs (arrows 5, 6), being addressed to RyR, ARC, and PKB. Owing to that, NOS-RG might have high sensitivity to this kind of cross-control. Corresponding examples will be

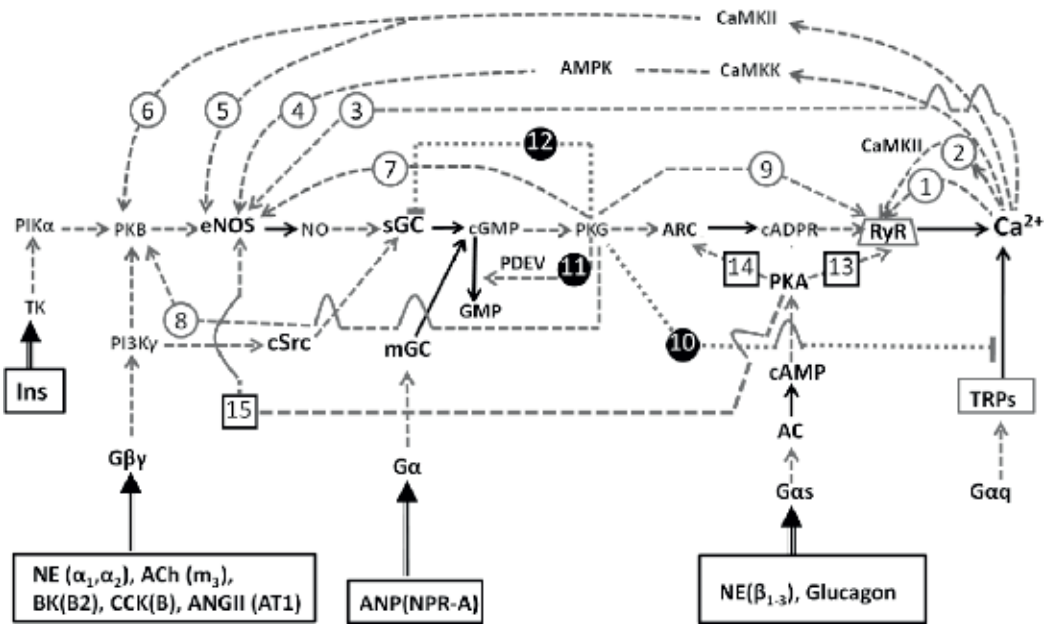

Figure 2. PI3K/PKB/eNOS/NO/sGC/cGMP/PKG1/ARC/cADPr/RyR/Ca^{2+}-signaling system with its system of autoregulation and cross-control by AC/cAMP/PKA-signaling pathways. All abbreviations and explanations are given in the text. Various types of activation and inhibition are indicated as broken arrows and dotted lines (with symbol T), correspondingly. The family of nested positive feedback loops (arrows with white circles) has the numbers from 1 to 8. Positive feedforward loop is numbered as 9. Negative feedbacks (marked by black circles) have the numbers 10 through 12. Crosstalk loops, describing positive impact of AC/cAMP/PKA-signaling pathway, have the numbers 13 through 15. Various hormones and neurotransmitters (with corresponding receptors), activating G-proteins and TKs, are placed in the boxes.

presented later. However, AC/cAMP/PKA-signaling pathway is under negative cross-control of NOS-RG (**Figure 1**). Robustness of NOS-RG and complexity of its crosstalk with AC/cAMP/PKA-signaling pathway represent serious problems in experimental studies and mathematical modeling of such systems.

2.3. Oscillatory and triggering regimes registered in adipocytes

Table 1 summarizes our earlier results, characterizing action of several hormones and neurotransmitters on Ca^{2+}-signaling systems of cultured epididymal adipocytes (9DIV) of white healthy mice. ACh, activating *m3*-muscarinic receptors (*m3-MR*), may elicit periodic Ca^{2+} oscillations in 70–80% of the cells. About 10–15% of the cells respond by spikes. Rest of the cells is silent. Applied inhibitory analysis indicates the implication of NOS-RG [61]. In contrast to Ach, NE by activating PLC-RG via *α1A-AR* evokes Ca^{2+} oscillations in 30–40% of the cells. Subsequent application of NE, after washing of cultured cells of Ach, may induce Ca^{2+} oscillations in the same percentage of cells. Fast monomodal or complex multimodal oscillations may be observed in dependence of cell size [57]. Two lines of numbers at the fifth row describe these two limits of oscillations periods, which were registered in cultures studied.

In comparison with ACh, ANP, and NE, peptide hormones insulin, Ang II, and BK (**Table 1**) may evoke rhythmic activity by involving first or second oscillatory mechanisms (PLC-RG or NOS-RG) in dependence of cellular culture used. Besides that, insulin, CCK, BK, and ANP may often elicit complex multiperiodic and chaotic Ca^{2+} oscillations.

Agonists	**ACh [61]**	**NE [51]**	**Ins [59]**	**Ang II [60]**	**ANP [58, 61]**	**CCK-4 [58]**	**BK ****
Receptors involved and concentrations of agonists used	m3	α1	TK	AT-1	NPR-A	CCK-B	B2R
	1-5 μM	1-5 μM	3-5 nM	300-500 nM	1-5 μM	1-10 nM	0.3-10 μM
PLC-RG, % of cells with rhythmic activity	—	30–40	20–30	20–30	—	20–30	30–40
NOS-RG, % of cells with rhythmic activity	70–80	—	15–25	30–35	30–40	20–40	25–30
Periods of oscillations (s) *	5–60	20–75	20–30	20–50	20–50	25–30	10–30
	100–300	100–300	50–150	75–200	200–300	300–500	200–500

In the table, second and third rows describe, which of two Ca^{2+} signaling systems is turned on by corresponding agonist. Numbers, presented at these rows, show average percentage of all cells in the cultures tested, which generate mono and multimodal oscillatory regimes, or chaotic oscillations. *Periods of minimal and maximal modes of oscillations, observed in the cells of different size, are presented in fourth row. In each experiment 5–10% of all cells were nonresponsive. Rest of the cells was characterized by Ca^{2+} spikes. From 10 to 15 experiments were used for each agonist applied. The number of monitored cells in each culture was 80–100 cells. References are indicated in the square brackets in top row. **Taken from: Turovsky et al., submitted to publisher.

Table 1. Involvement of two Ca^{2+}-signaling systems: PLC and eNOS-dependent robust generators (PLC-RG and NOS-RG) in rhythmic processes evoked by hormones and neurotransmitters in cultured epididymal adipocytes (9DIV) of white 4–6 weeks old male mice.

2.4. Some elements of the control of both Ca^{2+}-signaling systems

2.4.1. Control of IP3R by PKG1

Preliminary results, obtained with fluorescent antibodies staining, indicate smooth dense distribution of IP3R in adipocytes in comparison with smooth but thin distribution of RyR (Turovsky et al., submitted to publisher). This difference corresponds to substantial difference in mRNA expression of the subtypes of IP3R and RyR-receptor proteins (see below). Due to the expression of both types of Ca^{2+}-channels in adipocytes, we might expect their tandem operation under the action of ACh. However, preincubation of cultured cell with PLC or IP3R inhibitors does not alter ACh effects [61]. Moreover, combined application of PLC inhibitors and IP3R antagonists added after ACh may only diminish the amplitude of Ca^{2+} oscillations by10–15% [61]. All this may indicate that expected tandem operation of RyR and IP3R, or supportive role of IP3R [24, 26], is not realized due to inhibitory action of PKG1 on IP3R (**Figure 1**, dotted line 12) and, possibly, on PLCβ. Recent data, demonstrating endothelium-dependent suppression of AVP-evoked Ca^{2+} oscillations in microvessel's myocytes by ACh, support this conclusion [85]. Taken together, these results may indicate universal role of the control of IP3R by PKG1. To stress the question, we might also speculate that, in spite of low protein content of NOS-RG in adipocytes, high activity of this system is supported (reinforced) by unusual multiloop feedback control.

2.4.2. Robustness of NOS-RG: Impact of PFLs, based on activation of several targets by CaMKII and AMPK

CaMKII may be involved in the activation of RyR, eNOS, and PKB (arrows 3, 5, 6 at **Figure 1**). AMPK, being activated by Ca^{2+}-dependent CaMKIV, may also promote further activation of eNOS (arrows 4 at **Figure 1**). To break corresponding PFLs, we applied the inhibitors of both enzymes. To avoid nonspecific effects, we used low concentrations of the inhibitors, equal to their *Kd*. Our preliminary studies have shown that the applications of KN-63 (inhibitor of CaMKII) and of Compound C (inhibitor of AMPK) altered the shape of Ca^{2+}-oscillations and even suppressed rhythmic activity in part of the cells (**Figure 3**). Combined effect of both inhibitors was statistically significant ($p \leq 0.02$). Rather weak effect of Compound C on NOS-RG might be explained by the fact that the activation of AMPK by CaMKIV (at the conditions of our experiments) is insufficient to keep required gain of PFL(4) (arrow 4 at **Figure 2**). Besides CaMKIV, the activity of AMPK is controlled by AMP, sirtuins (SIRT1), liver kinase B1(LKB1), and several other kinases [86].

2.4.3. On the involvement of α1,2-AR and β1: 3- AR in the activation of PLC-RG and NOS-RG

Agonist of *α1-AR* phenylephrine (5–10 μM), like to NE (**Table 1**), evokes Ca^{2+} oscillations in 25–30% of the cells, implicating PLC-RG. Sustainability of these oscillations depends on store-operated Ca^{2+} entry into the cells [63].

Agonists of *α2-AR* guanabenz (10 μM) and L-arginine (5–10 mM) may elicit Ca^{2+} spikes in 35–50% of cultured cells, involving NOS-RG [55].

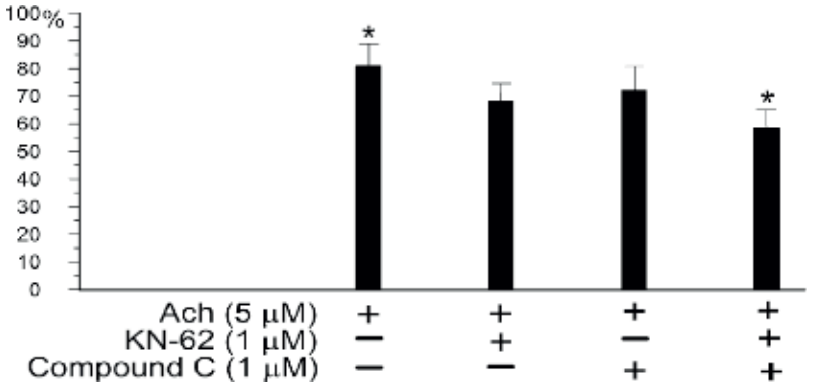

Figure 3. Robustness of NOS-RG. Impact of CaMKII and AMPK on Ca^{2+}- oscillations elicited by ACh in cultured adipocytes (9DIV). Bars represent average number of the cells, which respond to added ACh and the inhibitors of CaMKII (KN-63) and of AMPK (compound C). The inhibitors were added 10 min after the application of ACh. Details are given in the text. N = 6. Data presented as mean ± SD. *denotes statistically significant difference $p \leq 0.02$. Results are taken from: Turovsky et al., submitted to publisher.

Activation of *β1–3- AR* by isoproterenol (3–5 μM) is characterized by slow rise of Ca^{2+} in 40–50% of cells. The antagonists of IP3R and RyR suppress Ca^{2+} responses in 75–80% and 15–20% of activated cells, respectively [62]. Observed difference in this suppression may characterize the impact of PKA on the phosphorylation and activation of IP3R and RyR.

2.4.4. Signaling interplay and sensitivity: Synergistic action of low concentrations of ACh and NE

Low concentrations of ACh, NE, phenylephrine, and L-arginine cannot induce Ca^{2+} responses in adipocytes. However, sequential or combined application of ACh and these agonists display synergistic effects, promoting diverse oscillatory regimes (**Figure 4A–C**) and triggering oscillatory transitions from one stable steady state with low Ca^{2+} level to the second steady state with high Ca^{2+} concentration in the cell (**Figure 4B**, record 2) (Turovsky et al., in publication). This kind of synergy may be explained by combined action of various Gβγ- proteins on signaling axis (6): Gβγ → PI3Kγ → PKB → eNOS.

Combined action of low concentration of ACh and of isoproterenol may elicit complex oscillations (**Figure 4D**), triggering transition from one stable state to another (**Figure 4E**) and Hopf bifurcation, that is, transition from stable steady state to stable oscillatory regime (**Figure 4F**). The mechanism of synergistic action of ACh (*m3-MR*) and of isoproterenol (*β1–3 - AR*) may be based on the activation of axis (6) by ACh, reinforced by the activation of PKB (in this axis) and of ARC and RyR (in NOS-RG) by PKA (arrows 13–15 at **Figure 2**).

2.4.5. cADPr and RyR may play a supportive role in the operation of IP3R and PLC-RG

Nicotinamide (NAM), product and inhibitor of cADPr synthesis by ARC (or CD38), has some influence on Ca^{2+} oscillations evoked by NE. Added NAM (10 mM) may change the shape of oscillations in 20–30% of oscillating cells and suppress rhythmic activity in 15–20% of cells (Turovsky et al., submitted to publisher). This may indicate tandem operation of IP3R and RyR in some part of the cells, having rhythmicity evoked by NE.

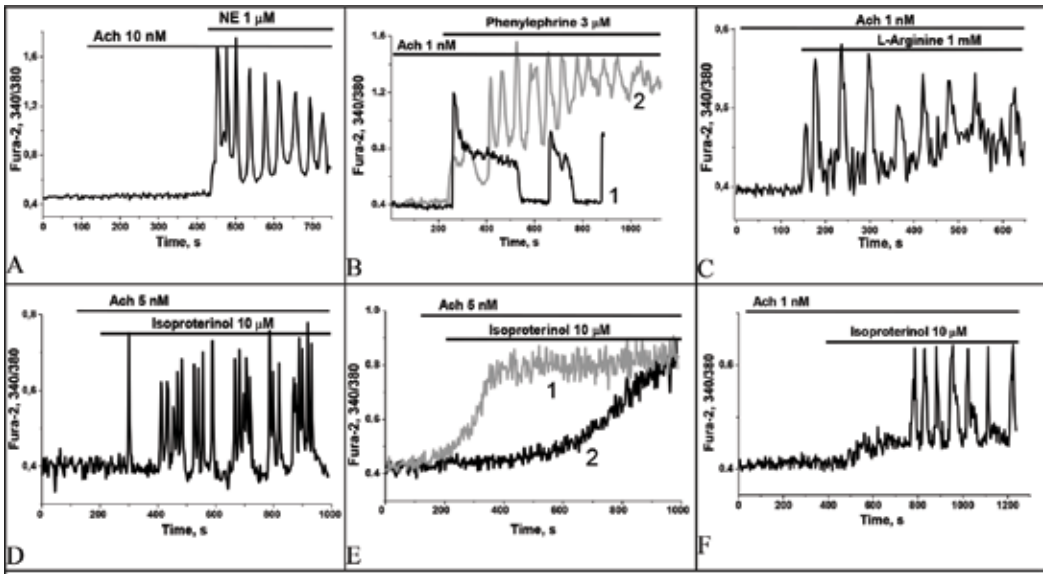

Figure 4. Signaling interplay and sensitivity. Synergistic action of low concentrations of ACh and NE. A–C: Interplay of Gβγ –subunits of G- proteins of GPCR. Ca^{2+}- oscillations and triggering regime (4B, record 2) produced by combined action of low concentrations of ACh and NE, ACh and phenylephrine, ACh and L-arginine, correspondingly. Record 1 at B represents an example of relaxation oscillations. D–F: Interplay of Gβγ- subunits of Gq- proteins (ACh) and of *β1–3 - AR* (isoproterenol). Complex oscillations (D), triggering regimes (E) and Hopf bifurcation (F) produced by combined action of low concentrations of ACh and isoproterenol. Description in the text. Results from: Turovsky et al., submitted to publisher.

3. Loss of rhythmic activity and suppression of mRNA expression for the proteins of Ca^{2+}- signaling systems at obesity

3.1. Influence of cell size on rhythmic activity of adipocytes of healthy animals

Preadipocytes isolated from healthy male mice, growing on high-glucose DMEM medium, become differentiated to the ninth day of culture (9DIV). Mature adipocytes represent heterogeneous populations of cells, which is characterized by different cell size, in dependence of number of lipid droplets inclusion [57]. We evaluated cell size by measuring the area (S) occupied by the cell. Small adipocytes, having S ≈ 300–400 μm^2, generated fast regular monomodal Ca^{2+}- oscillations with periods from 5 to 75 s in response to ACh or NE [57]. Such cells accounted for 10–15% of all monitored cells in culture and had few small lipid droplets. More than 50% of the cells had cellular size S ≈ 500–900 μm^2. Rest of cells (15–20%) with S ≤ 1100 μm^2 had several big lipid droplets or one lipid inclusion, which might occupy from 70 to 90% of the cell volume. ACh and NE elicited complex multimodal Ca^{2+} oscillations with periods from 100 to 300 s in the cells with S ≥ 600 μm^2. Similar results, characterizing correlation of cell size with the shape and period of oscillations, have been registered for insulin [59]. Results presented in lower part of **Table 1**(fifth row) indicate that, independently of agonist used, cultured cells may generate Ca^{2+} oscillations in the ranges of periods from 5–60 s to 400–500 s.

Rhythmicity disappeared in hypertrophied adipocytes with S ≥ 1200 μm^2, which may respond to the application of high doses of ACh or NE (20–30 μM) only by Ca^{2+} spikes or slow Ca^{2+} accumulation [57]. These observations may indicate that uncontrolled hypertrophy and corresponding cytoplasm shortage might predispose to loss of rhythmic processes in adipocytes and to the development of general hormonal resistance in WAT cells.

3.2. Impact of obesity on Ca^{2+} signaling systems of adipocytes

3.2.1. Model of obesity

We used 6 to 8 month course of high-fat feeding, based on the addition of pork lard (200–300 mg/day/animal) to standard chow of rodents, taking in experiments 7–8 month old mice. This model is described briefly in Appendix. Obese 6–7-month-old mice had elevated levels of glucose in blood in a fasted state (7–9 mM), raised arterial pressure (130–150 mm Hg), and macromolecular liver steatosis (Grishina et al., submitted to publisher).

3.2.2. Ca^{2+} signaling in hypertrophied primary adipocytes and cultured cells

Isolated primary epididymal adipocytes of medium size (S ≈ 6000–7500 μm^2) had approximately 1–5% of cytoplasm (**Figure 5B** and **C**), which looks like bright oreol around of adipocyte. Most of these cells, being attached to cover glass by Cell-Tak adhesive, cannot generate Ca^{2+} signal in response to added high concentrations of ACh (**Figure 5D**). However, most of hypertrophied cells, having spots of cytoplasm, still preserve the ability to respond to added Ca^{2+} (**Figure 5E** and **F**) or ionomycin (**Figure 5D**). This kind of nonresponsiveness to ACh might characterize general hormonal resistance of hypertrophied eWAT cells in obese state.

Preadipocytes isolated from fat pads of obese animals cannot grow on glucose, being adapted to use long-chain fatty acids (LCFA) incoming from the blood. Incubation of preadipocytes with 100 nM of L-palmitoylcarnitine (PC) and standard high-glucose DMEM provides required conditions for cell differentiation and lipid accumulation. ACh, NE, and insulin may evoke weak Ca^{2+} signals in some part of cultured cells. Oscillations and standard amplitude Ca^{2+} spikes have never been observed in this kind of cell (Turovsky et al., submitted to publisher). These radical alterations in Ca^{2+}-signaling and hormonal sensitivity, registered in cultured cells of obese animals, may depend on radical alterations of mRNA expression for the enzymes and channels of both Ca^{2+} signaling systems (PLC-RG and NOS-RG).

3.2.3. Impact of LCFA on mRNA expression of cultured cells

Our preliminary results, obtained with the application of real-time PCR analysis (see Appendix), revealed significant depression of mRNA expression in cultured adipocytes of obese animals in comparison with the cells, which have been isolated from healthy animals. Results presented in **Figure 6** (gray columns) demonstrate that cultures grown on the medium containing 100 nM of PC have 3–5 fold lowering of the expression of: Ca^{2+}-dependent genes (of NFAT, NF*k*B); genes of proteins involved in energy and lipid metabolism (citrate synthase (CS) and HSL); marker genes of SIRT1, AMPK, PI3Kγ, and of eukaryotic translation initiation factor 2 alpha kinase 3 (PERK). As for NOS-RG and PLC-RG, the expression of mRNA of eNOS (NOS3), CD38, and RyR3 had fallen 10 times or more. The gene of IP3R isoform 3 (IP3R3) was

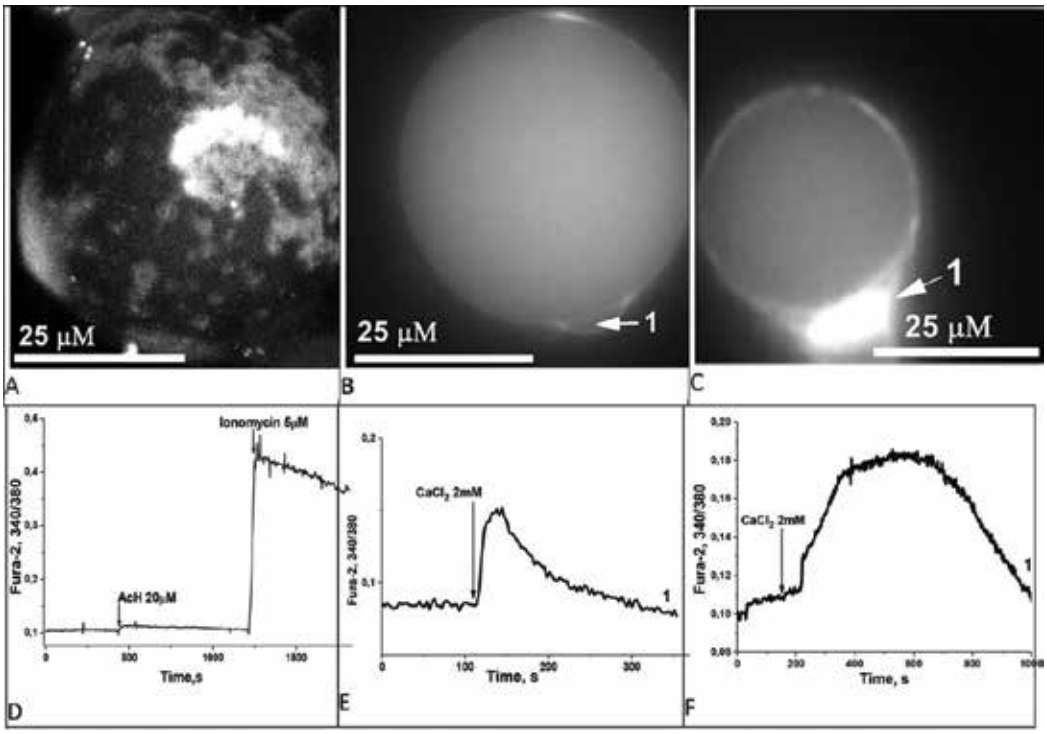

Figure 5. The shape and Ca^{2+} responses of primary hypertrophied adipocytes of obese mice. (A) confocal image of 3D reconstruction of primary hypertrophied adipocyte of obese mice (front view of one cell). The projection describes general uneven distribution of the cytoplasm in the cell loaded by Fluo-4. Fluorescent space corresponds to the part of cell occupied by the cytoplasm (up to 1–3% of total cell volume), while the bulk of the cell is engaged by fat droplet. (B, C) bright fluorescent areas around hypertrophied cells visualize the cytoplasm by fura-2 dye and are numbered as 1. Intracellular Ca^{2+} responses (Fura-2, ratio) from these areas, which were elicit by external Ca^{2+}, are presented at panels E and F, correspondingly. Panel D describes rare type of Ca^{2+} response evoked by ACh. Panel D also demonstrates ACh resistance of hypertrophied cell. Though, the response to ionomycin is preserved. From Sergeev et al., submitted for publisher.

most resistant to toxic action of very low dose of PC. The expression of inducible NOS (NOS2) mRNA was measured at the level of detection (Grishina et al., submitted to publisher).

Incubation of preadipocytes (isolated from obese animals) with 1 μM of PC revealed dramatic suppression of mRNA expression for all genes analyzed in cultured cells (9DIV). The expression of marker genes of NOS3 and RyR3 was not observed (marked in **Figure 6** as *). These results support widely distributed viewpoint on LCFA toxicity and indicate an important role of LCFA in the development of "adiposopathy". Observed radical alterations in mRNA expression, especially for the proteins involved in the functioning of NOS-RG and PLC-RG, may mean that earlier discussed mechanisms of autoregulation and cross-control of both Ca^{2+} signaling systems are being lost under chronic toxic action of low concentrations of LCFA. This state of both systems may be characterized as absolutely deregulated state.

3.2.4. Expression of mRNA in eWAT of obese animals

In comparison with fat pads of age-matched healthy animals, eWAT of obese male mice is characterized by considerable down-regulation of the expression for all genes examined

(**Figure 7**). These alterations have some qualitative similarities with the results observed in cultured cells, especially with respect to the changes of expression of marker genes for the proteins of NOS-RG. Fat pads of obese animals have more than 8–12 times lowered expression of PKG1, PKG2, and eNOS genes. The expression of genes for RyR2 and RyR3 was under the level of detection. Observed significant down-regulation of IP3R1 and IP3R2 genes may mean that both Ca^{2+} signaling systems are in deregulated states. Considering NOS-RG as the system, which integrates hormonal signals involved in the control of NO bioavailability, we may conclude that the application of insulin, NE, ANP, and so on might be ineffective to rise NO level and PKG1 activity in "sick" fat depots of obese animals.

3.2.5. Benefit and disadvantages of physical activity in the treatment of obesity

Taking into account the benefit of physical activity in the treatment of obesity, we have applied a very low-intensity treadmill running program (6 weeks, 10 min/day) to treat diabetic overweight mice (56.2+/− 5.7 g. wt) in combination with animals treatment with NaCl (control group) or complex preparation, addressed for the treatment of liver diseases and hepatic encephalopathy [88]. However, in a control group, 8 of 20 diabetic mice treated with NaCl have died within first week of adaptation period due to exercise intolerance. Survivors were characterized by marked improvement in blood glucose and lipid profiles and in liver mRNA expression of all genes examined (of PLC-RG and NOS-RG). In comparison with control group, all animals treated with complex preparation tolerated exercise program well and showed further improvement in blood lipid profiles and mRNA expression [89]. Taking all this into account, we might speculate that application of various exercise programs to treat obese patients should be combined with the use of some performance-enhancing drugs, or drugs addressed to support liver and cardiovascular system, and so on.

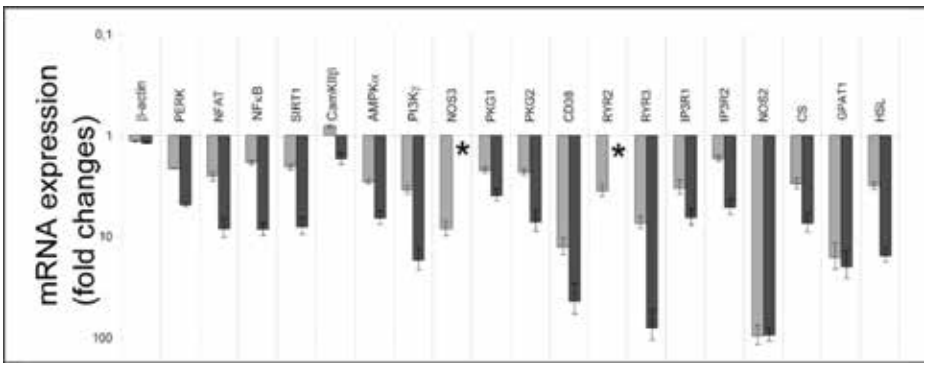

Figure 6. Down regulation of marker genes expression in cultured adipocytes of obese male mice grown in presence of L-palmitoylcarnitine. On the plot are resented: Ca^{2+}-dependent genes NFAT and NF*k*B, genes of PERK and proteins of NOS-RG, IP3R (IP3R1,2 - subtypes 1, 2), CamKIIβ, AMPKα, and of energy and lipid metabolism (citrate synthase (CS), GPAT1, HSL), β- actin and of inducible NOS (NOS2). Gene of GAPDH is used as reference gene. Mean expression in control adipocytes from healthy animals was set as 100%. Error bars indicate SD. Gray and black columns correspond to cultured adipocytes grown in presence of 100 nM and 1 μM of L-palmitoylcarnitine, correspondingly.* indicate the absence of mRNA expression for eNOS (NOS3) and of subtype 3 of RyR (RyR3). Horizontal line marked at the level 1 indicates baseline gene expression. N = 4, number of cultures in each group.

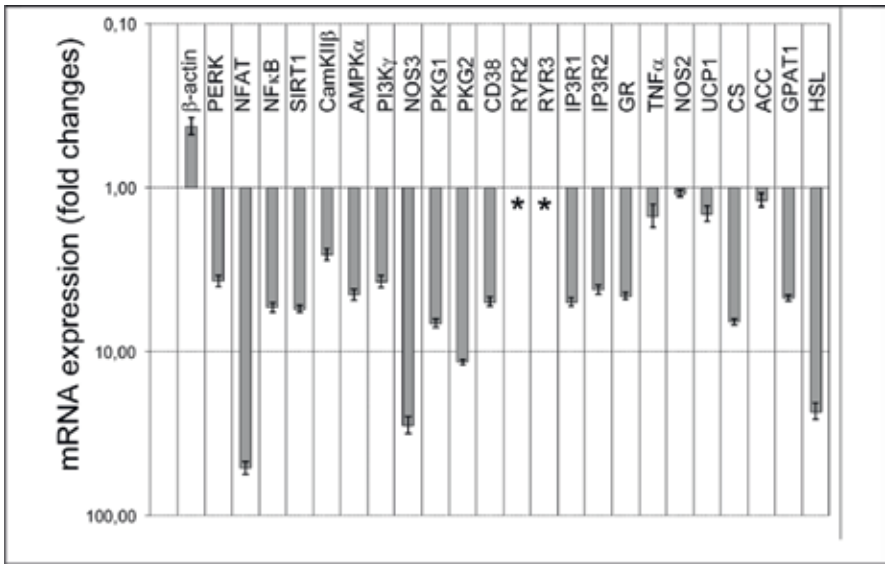

Figure 7. Down regulation of marker genes expression in eWAT of obese male mice. Presented Ca^{2+}-dependent genes NFAT and NF*k*B, genes of PERK and proteins of NOS-RG, IP3R (IP3R- subtypes 1, 2), glutathione reductase (GR), uncoupling protein 1 (UCP1), tumor necrosis factor α (TNFα), acetyl coenzyme a carboxylase (ACC), CamKIIβ, AMPK, and of energy and lipid metabolism (citrate synthase (CS), GPAT1, HSL), β-actin and NOS2. Gene of GAPDH is used as reference gene. Mean expression in control adipocytes from healthy 8 month old animals was set as 100%. Error bars indicate SD. N = 5, number of animals in each group.* indicate the absence of mRNA expression for both subtypes of RyR (RyR2, 3).

4. Conclusions

The main aim of our chapter was to reconstruct core elements of the Ca^{2+} signaling system of adipocytes and to demonstrate that this complex multivariable system cannot be divided on separate parts, independently controlled by various hormones and/or neurotransmitters. Having multiple feedbacks and cross-controls (**Figures 1** and **2**), this system makes interdependent the concentrations of all second messengers and the activities of various kinases. For example, considering lipogenic and antilipolytic action of insulin [7, 8], we have to take into account its lipolytic action. Insulin increases NO bioavailability and PKG1 activity by activating NOS-RG. The same effect may be produced by CCK, BK, Ang II (see **Table 1**). Reliability of NOS-RG, receptors' signaling interplay, and amplification of signals create important properties of Ca^{2+} signaling system, providing integration of hormonal signals at their low concentrations (**Figure 4**). This is especially important with respect to ACh.

Parasympathetic control of WAT is still under the question [90]. However, in our experiments ACh evokes marked Ca^{2+} and NO responses in cultured cells, implicating NOS-RG [61]. Some sensitivity to ACh is preserved in primary hypertrophied adipocytes ([57] and **Figure 5D**). Due to that, possible role of ACh in the control of WAT metabolism requires further investigations.

Gradual loss of rhythmic activity and the appearance of hormonal resistance, which are observed in hypertrophied cultured cells and in primary adipocytes isolated from obese animals, may be considered as markers of cell viability in the progress of pathologic process. Similar alterations in rhythmicity and resistance to ACh and NE have been registered in aorta rings isolated from obese and diabetic rats [87, 89].

Loss of rhythmicity in adipocytes is based on the alterations in enzyme activities and loss of feedback control of PLC-RG and NOS-RG, due to marked alterations in mRNA expression of corresponding genes (**Figures 6** and **7**). Our preliminary results indicate that qualitatively similar alterations in gene expression are observed in the liver of obese and diabetic mice [90].

All this may indicate universal mechanisms resulting in deregulation of metabolic and signaling systems in various organs and tissues.

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Conflict of interest

Authors declare no conflict of interests.

A. Appendix

Gene expression analysis

Gene expression in cultured adipocytes and in eWAT was performed using real-time PCR (Applied Biosystems 7300) with TagMan Universal Master Mix II, no UNG (Applied Biosystems). Total RNA was isolated with TRIzol (Invitrogen). RNA was quantified by Qubit® RNA BR Assay Kit (Molecular Probes, Eugene, OR) and cDNA was synthesized from 5 μg of total RNA using a reverse transcription system with random primers (Sileks, Russia). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH was used as reference gene. All results were normalized to GAPDH mRNA expression. Fold difference in each gene expression was calculated as $2^{-\Delta\Delta Ct}$. ΔΔCt were calculated relative to corresponding gene of control adipocytes grown on glucose, or of control healthy age-matched white male mice.

Animal model of obesity and type 2 diabetes

Animal model of obesity and type 2 diabetes (T2D), described for rats previously [87], was used in present experiments. This model is heterogeneous, like those presented by Duval et al. [5]. We used 6–8 month course of high-fat feeding, based on the addition of pork lard

(200–300 mg/day/animal) to standard chow of rodents, taking in experiments 7–8-week-old mice. Obese 6–7-month-old fat-responsive mice had elevated level of glucose in blood in fasten state (7–9 mM), raised arterial pressure (AP = 130–150 mm Hg) and macromolecular liver steatosis (Grishina et al., submitted for publisher). The animals with advanced T2D (9–10 month) have been characterized by: AP = 140–170 mm Hg, fasting glucose level of 12.1 ± 1.8 mM (SD), insulin of 2.9 ± 1.3 ng/ml (SD) and venous blood ammonia higher than 100–140 μM, liver fibrosis or even cirrhosis. Dysfunctional preadipocytes, isolated from "sick" epididymal fat depots of diabetic mice, were characterized by inability to proliferate (Turovsky et al., submitted for publisher).

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Chapter 12

Effect of Trans Fatty Acid on Insulin Responsiveness and Fatty Acid Composition of Lipid Species of 3T3-L1 Adipocytes

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Additional information is available at the end of the chapter

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Abstract

Trans fatty acids (TFAs) have at least one non-conjugate double bond in trans and TFAs are divided into two groups such as naturally or industry-occurring groups. Recent studies reveal that intake of industrial TFA is linked to increased risk of cardiovascular disease. Moreover, several studies suggest that intake of industrial TFA increases risk of diabetes, although other studies show that intake of industrial TFA is not associated with diabetes. Therefore, we used adipocytes which play important roles in glucose metabolism and development of diabetes, and our previous study showed that persistent exposure to elaidate, a major industrial TFA, impairs insulin-dependent glucose uptake of adipocytes. Since phospholipid acts as a scaffold for molecules of insulin signaling, we analyzed intracellular distribution of elaidate and fatty acid composition of lipid species. Incorporated elaidate is esterified into triglyceride and phospholipid. Moreover, elaidate-esterified phospholipids are distributed in various organelles. Intriguingly, persistent exposure to elaidate reduces the amount of oleate in phospholipid of mitochondria and plasma membrane and disturbs the equilibrium between bent and linear-shaped chain fatty acid. Therefore, disturbed equilibrium of fatty acid composition of phospholipid should be considered to elucidate the mechanism for impaired insulin responsiveness of adipocytes exposed to elaidate.

Keywords: elaidate, trans fat, insulin responsiveness, phospholipids

1. Introduction

1.1. Intake of trans fatty acids from daily diet

Trans fatty acids (TFAs) are defined as unsaturated fatty acids which have at least one non-conjugated double bond in the trans form [1] and are divided into naturally or industry-occurring group. A major naturally occurring TFA is vaccenic acid (*t*11-18:1), and it is produced by gastric bacteria of ruminants, for example, cow and goat [2]. Since meat and milk of ruminants contain vaccenic acid [3], we intake vaccenic acid from daily diet and vaccenic acid is contained in human blood [4]. On the other hand, a major industry occurring TFA is elaidate (*t*9-18:1), which is an artificially produced cis form of unsaturated fatty acids during hydrogenation of vegetable oil and food frying [5]. Therefore, we intake elaidate from shortening, margarine and fried food such as fried potato, snack food and instant noodles [6, 7]. It is reported that elaidate as well as vaccenic acid is contained in our blood at approximately 10–30 μM [4, 8].

1.2. Relationship between intake of industrial trans fatty acids and risk of cardiovascular disease and governmental regulation of dietary intake of trans fatty acids

It has been revealed that intake of TFAs-rich diet reduces HDL cholesterol level and raises LDL cholesterol level in healthy subjects [9]. Moreover, retrospective cohort study shows that intake of TFAs-rich diet is associated with higher risks of cardiovascular disease (CVD) [10]. These findings are supported by systematic review and meta-analysis [11, 12]. Furthermore, de Souza et al. also reveal that mortality of CVD is associated with intake of industrial TFAs, but not ruminant TFAs [11]. Consideration of those adverse effects of industrial TFAs on health, US Food and Drug Administration decided to eliminate industrial TFAs from food supply until 2018. Moreover, Denmark, Switzerland, Austria, Canada and Singapore have policy to limit or diminish industrial TFAs in food. Some other countries in Asia such as Korea and China obligate to labeling of content of TFAs. These governmental regulations reduce intake of industrial TFAs from daily diet, although industrial TFAs are contained in food in many countries [13, 14].

1.3. Is intake of trans fatty acids a risk factor for various diseases?

Emerging evidences suggest that intake of TFAs is associated with the development of various diseases. Systematic review by Barnard et al. indicates that intake of TFAs is associated with risk of cognitive disorders [15]. Moreover, Golomb et al. reports that dietary TFAs intake is negatively related to memory of the word in younger adults [16]. In addition, intake of TFAs is inversely related to sperm count in young healthy men [17]. However, since these studies do not distinguish between industrial TFAs and ruminant TFAs, it cannot be concluded that the adverse effects are caused by intake of industrial TFAs.

A systematic review by de Souza et al. also analyzes the effects of intake of TFAs on diabetes [11]. Intake of TFAs has no association with the risk of diabetes. However, intriguingly, when TFAs are divided into industrial and ruminant TFAs, intake of ruminant TFAs reduces the risk of diabetes. Although there is no data about industrial TFAs in the study, these results suggest

that intake of industrial TFAs elevates the risk of diabetes. This hypothesis is supported by in vitro study using rat fed with industrial TFAs-rich diet [18]. However, other groups report that industrial TFAs do not alter insulin responsiveness in epidemiological study [19], in vivo [20] and in vitro study [21]. Hence, the roles of industrial TFAs in development of insulin resistance and diabetes remain unclear.

1.4. Elaidate, an industrial trans fatty acid, impairs insulin responsiveness of adipocytes

What causes the differences in the effects of industrial TFAs on insulin resistance and diabetes? Osso et al. analyzed the effects of industrial TFAs for about 8 weeks [18]. On the other hand, Louheranta et al. and Lovejoy et al. analyzed for 2–4 weeks, respectively [19, 20]. Moreover, Granados et al. cultured 3T3-L1 adipocytes for 24 h in the presence of 100 μM elaidate [21]. We focused on the exposure period to industrial TFAs. To examine the effects of persistent exposure to industrial TFAs, 3T3-L1 preadipocytes were cultured and differentiated into adipocytes in the presence of 10 μM elaidate which is close to physiological concentration in human plasma [4, 8]. Persistent exposure to elaidate before and during differentiation impaired insulin-dependent glucose uptake and translocation of glucose transporter 4 (GLUT4) to the plasma membrane [22]. On the other hand, culture of 3T3-L1 adipocytes with 10 μM elaidate for 24 h did not alter insulin-dependent glucose uptake. Thus, our findings reveal new factor "period" for consideration of adverse effects of TFAs and emphasize the risk of persistent intake of industrial TFAs for development of diabetes.

1.5. How does elaidate impair insulin responsiveness of adipocytes?

Stimulation with insulin activates insulin-signaling cascades [23]. In brief, insulin binding to insulin receptor activates its kinase domain, and insulin receptor phosphorylates itself and insulin receptor substrates followed by activation of phosphoinositide 3-kinase (PI3K). PI3K converts phosphatidylinositol (3,4)-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3). PIP_3 acts as a scaffold of Akt at the plasma membrane, and it enables mTORC2 and PDK to phosphorylate and activate Akt. Activated Akt accelerates translocation of GLUT4 storage vesicles (GSVs) to the plasma membrane [24], and GLUT4 transports extracellular glucose into the cells. Our unpublished data indicate that insulin-dependent phosphorylation of Akt, but not insulin receptor, is suppressed in adipocytes persistently exposed to elaidate. Therefore, suppressive effects of elaidate on insulin signaling are partial but not overall. One possibility is that expression or activation of PI3K, mTORC2 or PDK1 may be repressed in adipocytes persistently exposed to elaidate. Another possibility is that alternation in fatty acid composition of phospholipids affects activation of Akt. It is reported that saturated fatty acid-contained phosphatidylcholine prevents full activation of Akt in vitro [25]. Moreover, oleate-contained PIP_3 enhances activation of Akt in vitro [26]. Thus, we should carefully analyze the effects of elaidate on insulin signaling around Akt.

Our previous data also indicate that incorporated elaidate may affect insulin responsiveness of adipocytes. Elaidate acts as a ligand and can activate G protein-coupled receptor 120 at several hours [27], although culture of adipocytes with 10 μM elaidate for 20 min or 24 h did not

alter insulin-dependent glucose uptake [22]. Therefore, ligand property of elaidate will not be involved in an impairment of insulin responsiveness. Another possibility is that incorporated elaidate impairs insulin responsiveness. Extracellular fatty acids are incorporated through FATP and CD36 [28, 29], and then fatty acids are converted into acyl-CoA derivatives by acyl-CoA synthetase [30]. Acyl-CoA derivatives are degraded by β-oxidation or esterified into lipid species such as triglyceride and phospholipid. It is reported that elaidate is esterified into triglyceride and phospholipid of heart of rats fed with elaidate-contained diets [31]. Moreover, elaidate is incorporated into adipose tissue of rabbits fed with elaidate-contained diets [32], although intracellular distribution of elaidate in lipid species of adipocytes is not fully understood. Therefore, we provide new data about intracellular lipid distribution of elaidate in adipocytes.

2. Incorporation of extracellular elaidate and its distribution in lipid spices of adipocytes

2.1. Materials and methods

2.1.1. Cell culture, differentiation and addition of fatty acids

3T3-L1 pre-adipocytes were purchased from Japanese Collection of Research Bioresources (Osaka, Japan). Cells were cultured in Dulbecco's modified Eagles medium containing 4.5 mg/L D-glucose (DMEM-high Glucose; Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, S1820; Biowest, Nuaillé, France), 50 units/ml penicillin and 50 μg/ml streptomycin (P7081; Sigma-Aldrich, St. Louis, MO). Before differentiation into adipocytes, cells were cultured for 10 days in the presence of 10 μM of bovine serum albumin (BSA)-conjugated elaidate (E4637; Sigma-Aldrich), stearate-d35 (BX-245; Olbracht Serdary Research Laboratories, Toronto, Canada), oleate-d17 (9000432; Cayman Chemical, Ann Arbor, MI), elaidate plus stearate (S4751; Sigma-Aldrich), elaidate plus oleate (O1382; Sigma-Aldrich) or BSA alone (vehicle), which was prepared as described [22]. Then, cultured 3T3-L1 pre-adipocytes were differentiated into adipocytes using 3-isobuthyl-1-methylxanthine (I7018; Sigma-Aldrich), dexamethasone (D4902; Sigma-Aldrich) and insulin (I5500; Sigma-Aldrich) for 8 days in the presence of BSA-conjugated fatty acids as described [22].

2.1.2. Subcellular fractionation

Subcellular fractionation was performed as described by Rangel et al. [33]. In brief, cells were washed twice with cold phosphate buffered saline (PBS) and once with lysis buffer (10 mM Tris-HCl, pH 7.4/250 mM sucrose/1 mM EDTA). Then, scraped cells were homogenized in lysis buffer with 7 mL-scale Dounce homogenizer (Kontes Glass Co, Vineland, NJ), and the homogenates were centrifugated at 1000 × *g* for 5 min with MX-107 (TOMY, Tokyo, Japan). The pellet was collected as nuclear fraction. The supernatant was further centrifugated with 20,000 × *g* for 20 min, and the pellet was collected as mitochondrial fraction. Then, the supernatant was ultracentrifugated at 100,000 × *g* for 60 min with Himac CS 150GX (Hitachi, Tokyo, Japan). The supernatant was collected as cytosolic fraction which will contain light vesicles,

and the pellet was collected as fraction of the plasma membrane. Each pellet was resuspended in PBS and sonicated for 10 s using a ultrasonic homogenizer (UR-21P; TOMY).

2.1.3. Analysis of fatty acids composition of triglyceride, free fatty acid and phospholipid

Total lipids were extracted from each fraction using a modified Bligh & Dyer method [34]. Triglyceride, free fatty acid and phospholipid in total lipids were separated with thin-layer chromatography (Silica gel 60, no. 1.05721.0001; Merck, Darmstadt, Germany) using development solvent (petroleum ether:diethyl ether:acetic acid = 82:18:1, v/v/v). Isolated triglyceride, free fatty acid and phospholipid was hydrolyzed and methylated using 10% toluene (no. 209-06791; Wako, Osaka, Japan) and hydrogen chloride-methanol reagent (5–10%) (X0038; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) for 1 h at 100°C. Then, amount of fatty acids in each lipid species was analyzed by GC-MS-QP2010 Ultra (SHIMADZU, Kyoto, Japan) as described [22]. Efficiency of extraction, isolation and methylation was normalized with triheneicosanoin (B-422; Olbracht Serdary Research Laboratories) for triglyceride, nonadecanoic acid (N5252-1G; Sigma-Aldrich) for free fatty acid and 1,2-diheptadecanoyl-sn-glycero-3-phosphorylcholine (no. 1400; Matreya LLC Inc., Pleasant Gap, PA) for phospholipid. The amount of fatty acids was also normalized with cell number.

2.2. Results and discussion

2.2.1. Extracellular elaidate is incorporated into adipocytes and esterified into triglyceride and phospholipid

To examine whether incorporated extracellular elaidate is esterified into lipid species of adipocytes, total lipid was extracted from adipocytes exposed to elaidate for 18 days, and it was separated by TLC followed by analyzing with GC-MS. In major lipid species of adipocytes was triglyceride, phospholipid and free fatty acid, and diacylglycerol and monoacylglycerol were in trace amounts. Therefore, we analyzed the amount of elaidate in triglyceride, phospholipid and free fatty acid and revealed that elaidate was contained in triglyceride and phospholipid (**Figure 1**). On the other hand, the non-esterified form of elaidate was not detected (**Figure 1**, free fatty acid). Moreover, the amount of elaidate in triglyceride was higher than that in phospholipid. These results suggest that incorporated extracellular elaidate is mainly esterified into triglyceride and phospholipid in adipocytes.

2.2.2. Elaidate-esterified phospholipid is distributed in various organelles membrane

Since phospholipids are a component of membranes of various organelles [35], we further examined intracellular distribution of elaidate-contained phospholipids using subcellular fractionation methods. Cells were homogenized and organelles in the lysates were enriched by centrifugation (**Figure 2A**). TLC and GC-MS analysis revealed that elaidate was contained in phospholipid of nuclear, mitochondrial, cytosolic and plasma membrane fraction (**Figure 2B**). The amount of elaidate in phospholipid of mitochondrial fraction was higher than that of nuclear, cytosolic and plasma membrane fraction, suggesting that elaidate-contained phospholipids are mainly distributed in mitochondria.

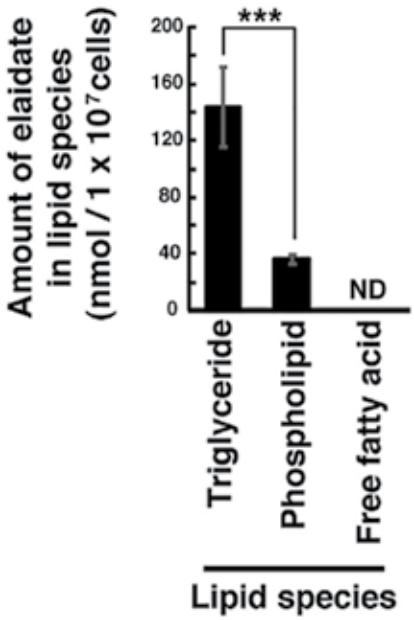


Figure 1. Extracellular elaidate is incorporated into adipocytes, and esterified into triglyceride and phospholipid. 3T3-L1 pre-adipocytes were cultured for 10 days and differentiated into adipocytes for 8 days with 10 μM elaidate. Amount of elaidate in triglyceride, free fatty acid and phospholipid were analyzed by GC-MS after separated by TLC as described in Section 2.1. Results are represented as means ± S.D., n = 5 independent experiments. ND means not detected. *Asterisks* indicate a significant difference (***$p < 0.001$) calculated by Student' s *t*-tests.

2.2.3. Amount of elaidate-contained phospholipids in organelles is different from that of stearate or oleate-contained phospholipids

Is the intracellular distribution of elaidate-contained phospholipids specific to elaidate or not? Therefore, we compared elaidate (trans-C18:1) with stearate (C18:0) which has similar steric structure to elaidate, or oleate (cis-C18:1) which is cis form of elaidate (**Figure 3A**). For trace of incorporated extracellular stearate and oleate, we used deuterium-labeled stearate (stearate-d35) and oleate (oleate-d17). As shown in **Figure 3B**, incorporated extracellular stearate-d35 (dark gray bars) and oleate-d17 (light gray bars) were esterified into phospholipid of each organelle, and stearate-d35 or oleate-d17-contained phospholipids were mainly distributed in the mitochondria fraction. It is reported that extracellular [^{3}H]-raveled arachidonate is incorporated into cells and the distribution ratio of [^{3}H]-raveled arachidonate in the mitochondria is lower than that in other organelles at several minutes after addition of [^{3}H]-raveled arachidonate into the medium [36]. However, the distribution ratio of [^{3}H]-raveled arachidonate dramatically rises at 24 h. Since cells were exposed to fatty acid for 18 days, incorporated elaidate, stearate-d35 and oleate-d17 may be transferred from other organelles to the mitochondria. Therefore, the mitochondrial distribution of elaidate is not specific to elaidate.

Intriguingly, although the amount of elaidate was similar to that of stearate-d35 and higher than that of oleate-d17 in the nuclear, mitochondrial and cytosolic fraction, the amount of elaidate in the plasma membrane fraction was significantly higher than that of both stearate-d35

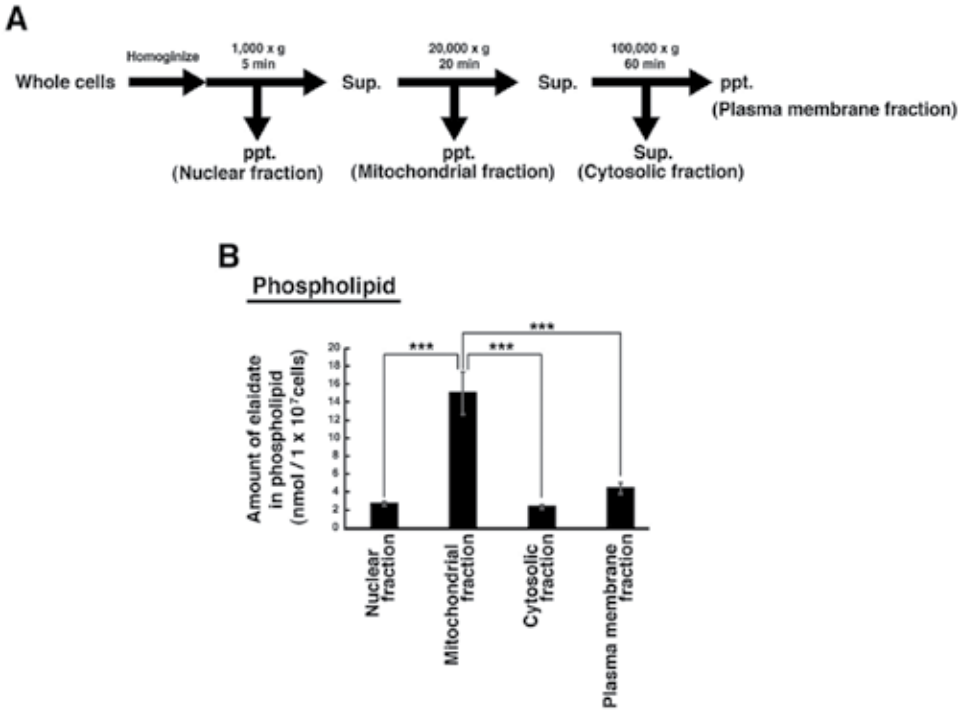


Figure 2. Elaidate-esterified phospholipid is distributed in various organelles membrane. 3T3-L1 pre-adipocytes were cultured and differentiated into adipocytes as described in **Figure 1**. (A) Each fraction was prepared by centrifugation from cell lysates. (B) Amount of elaidate in phospholipids of each fraction was analyzed using GC-MS. Results are represented as means ± S.D., n = 3 independent experiments. *Asterisks* indicate a significant difference (***$p < 0.001$) calculated by Student' s *t*-tests.

and oleate-d17. What causes the difference in the amount of these fatty acids in organelles? As shown in **Figure 3C**, the total amount of elaidate in phospholipid was higher than that of oleate-d17, but not stearate-d35. There were several possibilities that oleate-d17 was degraded through β-oxidation pathway, the amount of incorporated oleate-d17 into adipocytes was lower than that of elaidate, and oleate-d17 tend to be esterified into other lipid species such as triglyceride or to be removed from phospholipid by phospholipases compared with elaidate. It is reported that β-oxidation rate of oleate is higher than that of elaidate in rat heart homogenate [37]. Moreover, our unpublished data showed that the amount of oleate-d17 in triglyceride is higher than that of elaidate at 6 h after addition of those fatty acids into the culture medium of adipocytes, although there was no difference in total amount of oleate-d17 and elaidate. Since oleate and elaidate have a different steric structure, it may result in a difference between oleate and elaidate in metabolism and esterification into or removal from phospholipid.

Although steric structure of elaidate is similar to that of stearate, there was a difference in the amount of elaidate and stearate-d35 in the plasma membrane fraction (**Figure 3B**). Moreover, the amount of stearate-d35 was not significantly but slightly low in the mitochondrial fraction and high in the nuclear and cytosolic fraction compared with that of elaidate (**Figure 3B**).

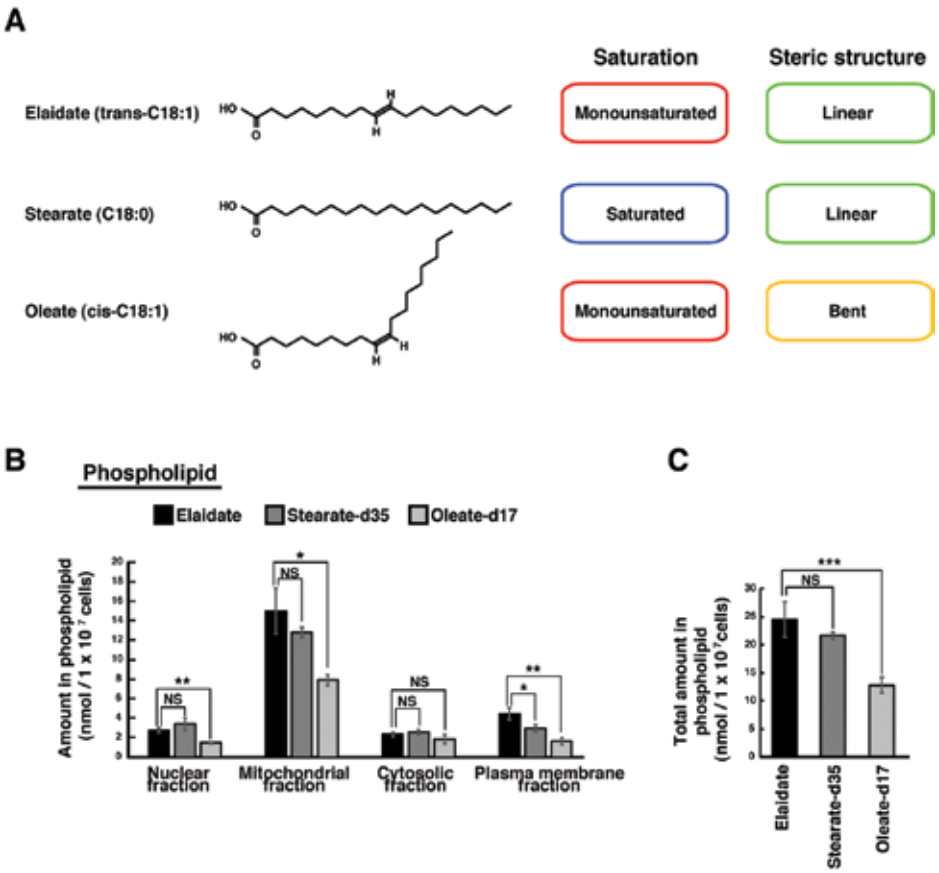


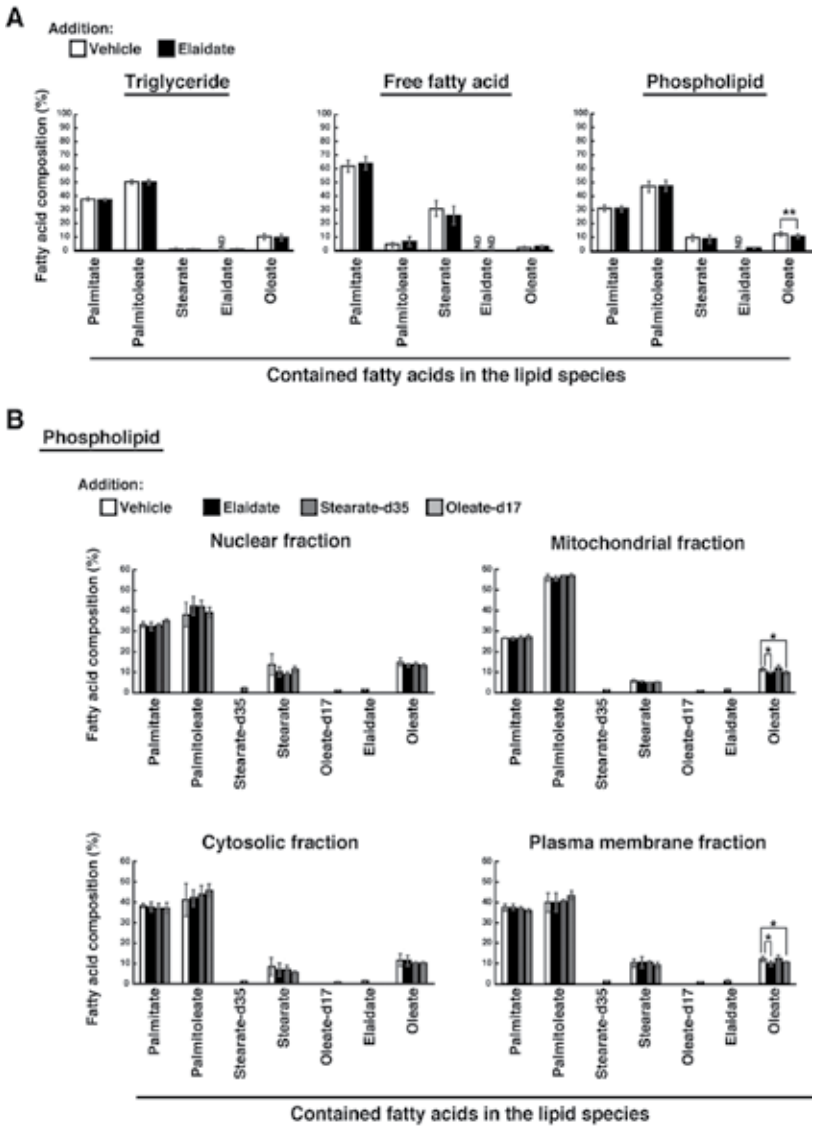
Figure 3. Amount of elaidate-contained phospholipids in organelles is different from that of stearate or oleate-contained phospholipids. (A) Structure of elaidate, stearate and oleate. (B) 3T3-L1 pre-adipocytes were cultured for 10 days and differentiated into adipocytes for 8 days with 10 μM elaidate (black bar), stearate-d35 (dark gray bar) or oleate-d17 (light gray bar). Amount of elaidate, stearate-d35 or oleate-d17 in phospholipid of each fraction were analyzed using GC-MS. (C) Total amount in phospholipids was calculated by summing up the amount of elaidate, stearate-d35 or oleate-d17 in each fraction. Results are represented as means ± S.D., n = 3 independent experiments. *Asterisks* indicate a significant difference (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS, not significant) calculated by Student' s *t*-tests.

It is known that stearate is desaturated by stearoyl-CoA desaturase-1 (SCD-1; n-9 desaturase) and converted into oleate [38]. We detected that exposure to stearate increases the amount of oleate but not stearate in adipocytes (unpublished data), suggesting that stearate is converted into oleate in adipocytes. Since elaidate is already desaturated at n-9 position, elaidate may be less likely to be metabolized by SCD-1. However, the different amount of elaidate and stearate in phospholipid was not only explained by the difference in the metabolism, suggesting that there are differences between elaidate and stearate in esterification into or removal from phospholipid. Therefore, the differences in both metabolism and esterification into or removal from phospholipid may result in different amounts of elaidate and stearate in phospholipid of organelles.

Thus, since elaidate has a double bond but exhibits linear-shaped structure, it may cause unique distribution of elaidate in phospholipid of various organelles membrane.

2.2.4. Fatty acid composition of phospholipid is altered in adipocytes exposed to elaidate

Next, we examined whether incorporation and esterification of elaidate into lipid species may disturb the equilibrium state of metabolism or distribution of other fatty acids. Therefore, we analyzed the amount of palmitate, palmitoleate, stearate and oleate, which were mainly contained in total lipid of adipocytes and calculated fatty acid composition (%). On the other hand, the amount of linoleate (C18:2), linolenate (C18:3) and arachidonate (C20:4) may be trace amount in 3T3-L1 adipocytes and we could not detect these fatty acids. As shown in **Figure 4A**, there was no difference in the fatty acid composition of triglyceride and free fatty acid. On the other hand, the percentage of oleate in phospholipid was slightly but significantly low in adipocytes exposed to elaidate compared with vehicle (**Figure 4A**).



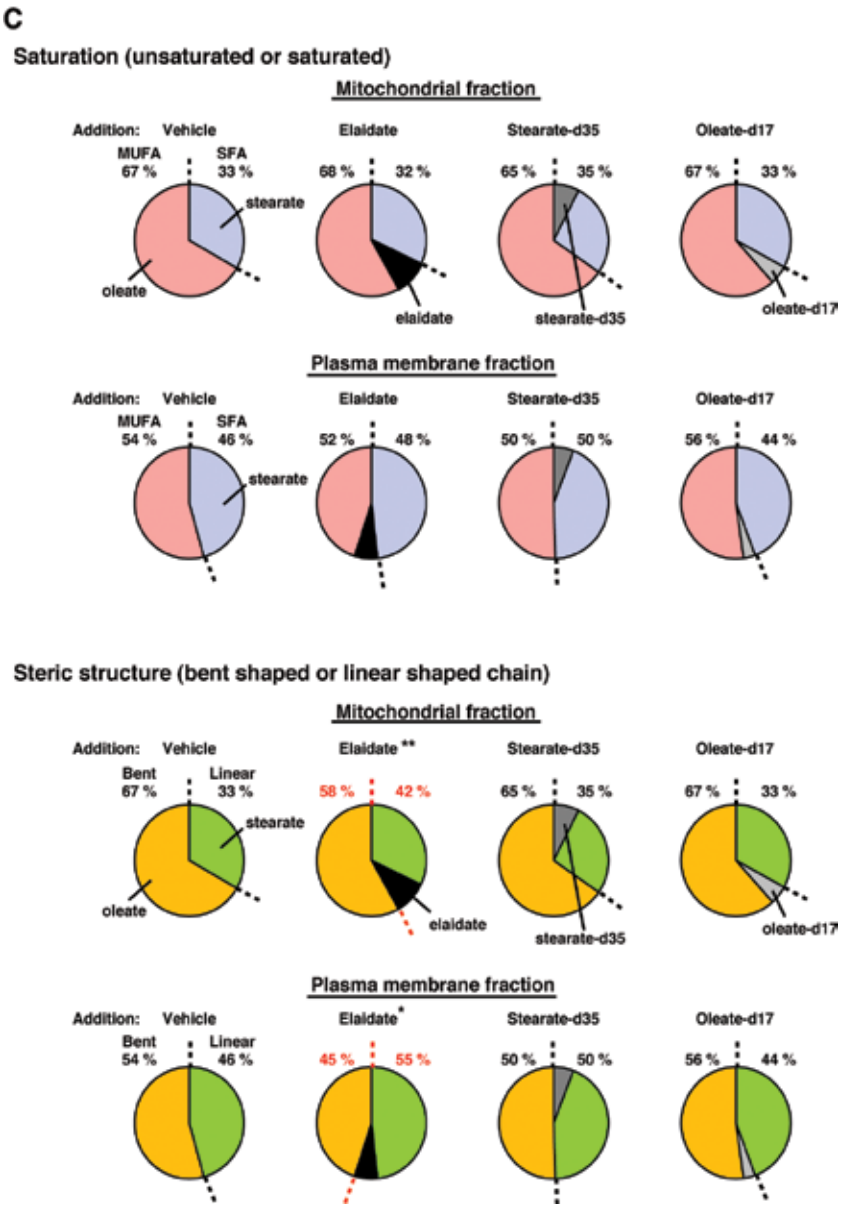


Figure 4. Fatty acid composition of phospholipid is altered in adipocytes exposed to elaidate. (A) Analysis of fatty acid composition of lipid species in adipocytes cultured with vehicle (white bars) or elaidate (black bars). Fatty acid composition (%) was calculated by the amount of each fatty acid dividing by total amount of palmitate, palmitoleate, stearate, elaidate and oleate. Results are represented as means ± S.D., n = 5–8 independent experiments. ND means not detected. (B) Analysis of fatty acid composition of phospholipid in organelles in adipocytes cultured with vehicle (white bars), elaidate (black bars), stearate-d35 (dark gray bars) or oleate-d17 (light gray bars). Fatty acid composition (%) was calculated by the amount of each fatty acid dividing by total amount of palmitate, palmitoleate, stearate-d35, stearate, oleate-d17, elaidate and oleate. Results are represented as means ± S.D., n = 3 independent experiments. (C) Fatty acid equilibrium (%) between monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) or bent shaped and linear shaped chain fatty acid in 18-carbon fatty acid. Results are represented as means, n = 3 independent experiments. *Asterisks* indicate a significant difference (*$p < 0.05$; **$p < 0.01$) calculated by Student' s *t*-tests.

Further analysis with subcellular fractionation revealed that the percentage of oleate was low in phospholipid of mitochondrial and plasma membrane fraction in adipocytes exposed to elaidate (**Figure 4B**, compare white bars with black bars). Moreover, this alteration was observed in both mitochondrial and plasma membrane fraction of adipocytes differentiated with oleate-d17 but not stearate-d35 (**Figure 4B**, see dark and light gray bars). Since there was no difference in the percentage of 16-carbon fatty acids (palmitate and palmitoleate), we surmised that elaidate limitedly affects the equilibrium of 18-carbon fatty acids. The equilibrium between monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) in 18-carbon fatty acids was maintained in adipocytes exposed to elaidate, stearate-d35 and oleate-d17 in both mitochondrial and plasma membrane fraction (**Figure 4C**, upper panels). However, when the steric structure was considered, elaidate has similar linear-shaped structure to stearate, and raises the percentage of linear-shaped chain fatty acid (**Figure 4C**, lower panels). Thus, in phospholipid of adipocytes exposed to elaidate, the equilibrium between bent and linear-shaped chain fatty acid was disturbed, although the equilibrium between MUFA and SFA was maintained.

The alteration was small compared with the total amount of fatty acid in phospholipid. However, since activation of insulin signaling occurs in the local area of the plasma membrane such as raft [39], elaidate may locally disturb the equilibrium of fatty acid of phospholipid and partially suppress the insulin signaling.

2.2.5. Elaidate does not compete with oleate

How does incorporated and esterified elaidate affect the equilibrium of fatty acid of phospholipid? There were possibilities that elaidate competes with incorporation of extracellular oleate into adipocytes or esterification of oleate into phospholipids, or elaidate indirectly reduces the amount of oleate through alteration of synthesis or metabolism of intracellular oleate. To examine the possibility of competitive inhibition, cells were exposed to 10 μM elaidate plus 1 to 10 μM oleate, which was expected oleate to competitively inhibit incorporation or esterification of elaidate into phospholipid and reduce the amount of elaidate. However, there was no difference in the amount of elaidate between cells exposed to elaidate and elaidate plus oleate (**Figure 5**). Since incorporated extracellular oleate and elaidate may exhibit different intracellular dynamics (see Section 2.2.3), elaidate does not compete with extracellular oleate in an incorporation into adipocytes or esterification into phospholipid. These results suggest that elaidate may alter synthesis or metabolism of intracellular oleate which results in a reduction of the amount and percentage of oleate in phospholipid.

Intracellular oleate is synthesized from stearate by SCD-1, and it is reported that SCD-1 prefers stearate than palmitate [40]. Therefore, reduced activity of SCD-1 may lead to reduced amount of oleate but not palmitoleate. However, it may raise the amount of stearate, although persistent exposure to elaidate did not alter the amount and percentage of stearate (**Figure 4**). Since the amount of palmitate and palmitoleate in triglyceride was significantly high in adipocytes exposed to elaidate compared with control (data not shown), reduced supplementation of palmitate from triglyceride to phospholipid may result in the prevention of increased amount of stearate, and maintain the equilibrium of MUFA and SFA, but not bent and linear-shaped chain fatty acid.

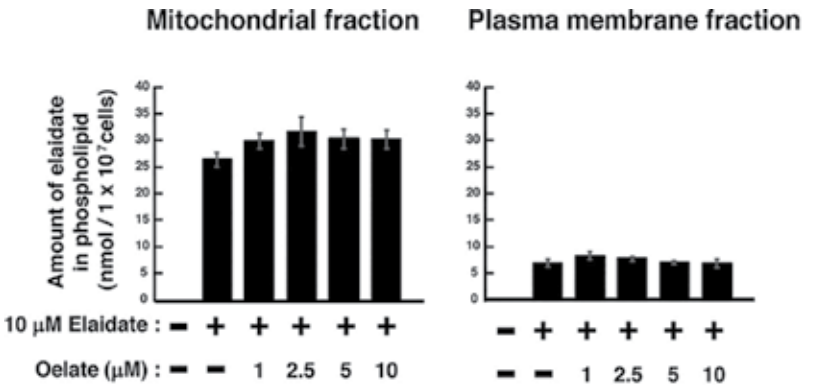


Figure 5. Elaidate does not compete with oleate. The amount of elaidate in phospholipid of mitochondria and plasma membrane fraction in cells differentiated with 10 μM elaidate plus 1, 2.5, 5 or 10 μM oleate. Results are represented as means ± S.D., n = 3 independent experiments. Significant difference was calculated by Student' s *t*-tests.

2.2.6. Intracellular distribution of elaidate and its effects on fatty acid composition of lipid species

Our results were illustrated in **Figure 6**. In adipocytes, incorporated extracellular elaidate is mainly esterified into triglyceride. The rest is esterified into phospholipid of various organelles such as mitochondria, plasma membrane, nucleus and cytosolic vesicles. Moreover, distribution of elaidate into phospholipid is not competitively inhibited by oleate and stearate (data not shown), suggesting that elaidate may exhibit unique intracellular dynamics or incorporation into cells is not a rate-limiting step. Furthermore, elaidate reduces the amount of oleate which results in the reduction of the percentage of oleate in phospholipid of mitochondria and plasma membrane. Intriguingly, esterification of elaidate and reduction of oleate disturbs the equilibrium between bent and linear-shaped chain fatty acid in phospholipid but not unsaturated and saturated fatty acid. Therefore, elaidate-esterified phospholipid may be recognized as oleate-esterified phospholipid.

To clarify the molecules which may recognize elaidate as oleate-esterified phospholipid and command to maintain the equilibrium of MUFA and SFA, we should separate phospholipid classes such as phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol and analyze its fatty acid composition, since enzymes which are involved in synthesis and metabolism of phospholipids (e.g. acyl-CoA synthases, acyltransferases and phospholipases) have substrate specificity to lipid classes and its fatty-acyl chain [41]. Thus, the maintenance of the equilibrium between MUFA and SFA in our results cannot be explained without a recognition system of fatty acids. Therefore, further analysis also enables us to elucidate the recognition system of fatty acids.

Note that certain phospholipids such as phosphatidylinositol and phosphatidylcholine have the potential to affect insulin signaling [25, 26]. Further analysis of alteration of fatty acid composition in phospholipid classes will lead to elucidate the mechanism for suppression of the insulin signaling.

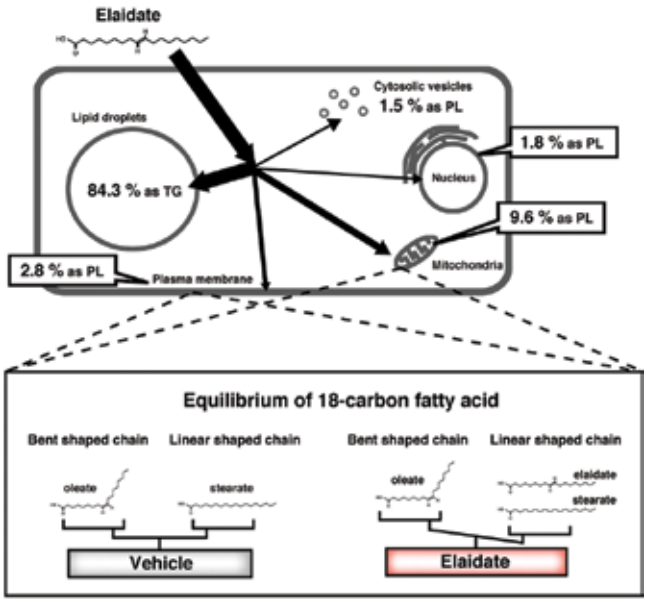


Figure 6. Intracellular distribution of elaidate and its effects on fatty acid composition of lipid species. Incorporated extracellular elaidate is esterified into triglyceride (TG) and phospholipid (PL) of organelles. Elaidate reduces the amount of oleate in phospholipid of mitochondria and plasma membrane, and disturbs the equilibrium between bent shaped and linear shaped chain fatty acid in phospholipid.

3. Conclusions

Our recent study reveals the deteriorative effects of elaidate on insulin responsiveness of adipocytes. In this chapter, we show for the first time that elaidate alters fatty acid composition of lipid species of adipocytes. Since lipid species may have important roles in regulation of insulin signaling, we should consider the effects of elaidate on lipid species to analyze the mechanism for an impairment of insulin responsiveness.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this chapter.

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Adipose tissue is a specialized connective tissue which, depending on type, plays different and significant roles in the human body: protects against environmental factors, stores lipids and triacylglycerol, synthesizes fatty acids and is involved in the process of thermogenesis. It is also a major secretory organ and highly active metabolic tissue. It secretes, for example, cholesterol, retinol, steroid hormones, prostaglandins and proteins known as "adipokines". Some of these molecules may be associated with pathologies such as obesity and insulin resistance. In humans, there are two main sites of adipose tissue accumulation: visceral and subcutaneous. Obesity is a worldwide health problem. This book also discusses a series of up-to-date topics about this pathology.

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