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Edited by Wilbert S. Aronow

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Published in London, United Kingdom







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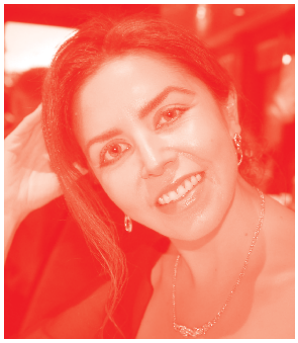

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http://dx.doi.org/10.5772/intechopen.87280
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Contributors
Olga Cvijanović Peloza, Sanja Klobučar Majanović, Dijana Detel, Gordana Kenđel Jovanović, Miro Bakula, Dario Rahelic, Tea Čaljkušić Mance, Sanja Zoričić Cvek, Bose Etaniamhe Orimadegun, Mezgebu Legesse Habte, Etsegenet Assefa Beyene, Teka Obsa Feyisa, Fariba Ahmadizar, Aniruddha Singh Singh, Megan Smith, Travis Huffman, Antonina Dunina-Barkovskaya, Drew Thomas, Darma Marcelin, Shone Almeida, Dinara Sadykova, Liliya Galimova, Evgeniia Slastnikova, Zulfiya Khabibrakhmanova, Natalia Guseva, Sugini, Mariana Suarez Bagnasco, Marité Cárdenas, Sarah Waldie, Rita Del Giudice, Kan Wang

First published in London, United Kingdom, 2021 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Management of Dyslipidemia
Edited by Wilbert S. Aronow
p. cm.
Print ISBN 978-1-83968-507-1
Online ISBN 978-1-83968-508-8
eBook (PDF) ISBN 978-1-83968-509-5

Meet the editor

Wilbert S. Aronow MD is Professor of Medicine and Director of Cardiology Research at Westchester Medical Center and New York Medical College. He has edited 19 books and is the author or co-author of 1,817 papers or book chapters, 752 commentaries, 50 Letters to the Editor, and 1166 abstracts. He has been a member of 185 editorial boards of medical journals. He has been a member of four national guidelines committees. He was a co-author of a 2015 position paper from the International Lipid Expert Forum. He has been a member of the Board of Directors of the ASPC, a committee member of numerous professional societies, and a consultant to many government agencies.

Contents

Preface

Dyslipidemia is a major risk factor for cardiovascular events, cardiovascular mortality, and all-cause mortality. The earlier in life dyslipidemia is treated, the better the prognosis. Treatment of dyslipidemia should follow the recommendations of the American Heart Association/American College of Cardiology lipid guidelines published in Circulation 2019; 139: e1046-e1081, and the recommendations of the European Society of Cardiology/European Atherosclerosis Society published in the European Heart Journal 2020; 41: 111-188.

The current book is an excellent one on dyslipidemia written by experts on this topic. This book includes 12 chapters including 5 on lipids, 4 on hypercholesterolemia in children, and 3 on the treatment of dyslipidemia. This book should be read by all health care professionals taking care of patients, including pediatricians since atherosclerotic cardiovascular disease begins in childhood.

I would like to thank all the contributors to this book for their excellent chapters. I would also like to thank Jasna Božić, the author service manager from IntechOpen, for her excellent assistance in editing this book.

Wilbert S. Aronow, MD, FACC, FAHA, FASPC, FACP
Professor of Medicine and Director of Cardiology Research,
Department of Cardiology,
Westchester Medical Center,
New York Medical College,
Valhalla, NY, USA

Section 1

Lipids

Chapter 1

Dyslipidemia: Current Perspectives and Implications for Clinical Practice

Sanja Klobučar Majanović, Olga Cvijanović Peloza, Dijana Detel, Gordana Kenđel Jovanović, Miro Bakula, Dario Rahelic, Tea Čaljkušić Mance, Tamara Šoić Vranić and Sanja Zoričić Cvek

Abstract

Dyslipidemia refers to a broad spectrum of various genetic and acquired disorders that affect blood lipid levels and largely contribute to global cardiovascular disease burden. Consistent evidence from epidemiological and clinical studies, supports the key role of the circulating LDL-cholesterol and other apoB containing lipoproteins in atherogenesis. All ApoB-containing lipoproteins with size less than 70 nm can cross the endothelial barrier, particularly in the presence of endothelial dysfunction. Uptake and accumulation of apoB-containing lipoproteins in the arterial wall is a critical initiating event in the development of atherosclerosis. Statin treatment, targeting LDL cholesterol reduction, remains the cornerstone of dyslipidemia management. There are abundant data supporting the concept of 'the lower LDL-C, the better' in the primary and secondary cardiovascular disease prevention. This chapter provides an overview of the key insights into the lipid abnormalities associated with an increased risk of CV events particulary in the context of dyslipidemia management in everyday clinical practice. Understanding the important role that metabolic derangements play in the pathogenesis of atherosclerosis pave the way for stronger implementation of current guidelines for CVD risk assessment and prevention.

Keywords: atherosclerosis, dyslipidemia, cardiovascular disease, lipid-lowering therapies, lipoproteins

1. Introduction

Cardiovascular disease (CVD) remains a major cause of global mortality and rising health care costs worldwide. CVD burden is predominantly attributable to modifiable behavioral and metabolic risk factors with dyslipidemia being one of them [1, 2]. Dyslipidemia is a term that encompasses a broad spectrum of various genetic and acquired disorders that affect blood lipid levels. This chapter provides an overview of the key insights into the lipid abnormalities associated with an increased risk of CV events particularly in the context of dyslipidemia management in everyday clinical practice. Understanding the important role that

metabolic derangements play in the pathogenesis of atherosclerosis paves the way for stronger implementation of current guidelines for CVD risk assessment and prevention.

2. Lipids and lipoproteins

Lipids are essential components of the human body, having several important biological functions such as storing energy, acting as structural components of cell membranes and participating in signaling pathways. The three main types of lipids are phospholipids, sterols, and triglycerides (also known as triacylglycerols) [3]. In view of the fact that the term "lipid" has been defined as any of a group of organic compounds that are insoluble in water but soluble in organic solvents, lipids comprise a broad range of molecules such as fatty acids, triglycerides (TG), phospholipids, sterols, sphingolipids and many others. However, from a clinical standpoint, given their role in the pathogenesis of CVD, the two major forms of circulating lipids in the body are TG and cholesterol. Although insoluble in plasma, these lipids can be transported throughout the bloodstream as lipoproteins when packaged with phospholipids and proteins known as apoproteins or apolipoproteins.

Lipoproteins are complex particles that have a central hydrophobic core composed of non-polar lipids, primarily cholesterol esters (CE) and TG and a hydrophilic surface consisting of polar lipids (phospholipids and free cholesterol) and apoproteins. The protein component provides structural integrity to the framework of the lipoproteins and being attached to the surface of particles, make them detectable for enzymes and receptors. Hence, apoproteins modulate enzyme activity (eg, apoprotein C-II activates lipoprotein lipase) and serve as ligands, specific recognition sites for cell surface receptors during cellular uptake (eg, apoprotein B-100 binds to the low-density lipoprotein receptor).

Lipoproteins are synthesized in both the liver and the intestines, playing a key role in the absorption and transport of dietary lipids by the small intestine, in the transport of lipids from the liver to peripheral tissues, and from peripheral tissues to the liver and intestine (a process known as reverse cholesterol transport). Within the circulation, lipoproteins go through constant change in composition and physical structure as the peripheral tissues take up the various components before the remnants return to the liver [4].

3. Classification and composition of plasma lipoproteins

Lipoproteins vary in size, density and composition which affects their functions, atherosclerotic risk profiles and other effects on health [3]. Based on major lipid and apolipoprotein content which determines their density, lipoproteins are classified into six categories; chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), lipoprotein (a) [Lp(a)], and high-density lipoprotein (HDL) (**Table 1**). Accompanying apolipoproteins and their functions are described in **Table 2**.

The main function of chylomicrons is the transport of dietary triglycerides from the intestine to the liver and other peripheral tissues, while VLDL particles carry endogenously synthesized triglycerides from the liver to other tissues. LDL particles are the major carriers of cholesterol in the circulation, supplying it to the cells, whereas the role of HDL is to transfer cholesterol from peripheral tissues to the liver.

Lipoprotein	Density (g/mL)	Diameter (nm)	TGs (%)	Cholesteryl esters (%)	PLs (%)	Cholesterol (%)	Apolipoproteins	
							Major	Others
Chylomicrons	<0.95	80-100	90-95	2-4	2-6	1	ApoB-48	ApoA-I, II, IV, V
VLDL	0.95-1.006	30-80	50-65	8-14	12-16	4-7	ApoB-100	ApoA-I, C-II, C-III, E, A-V
IDL	1.006-1.019	25-30	25-40	20-35	16-24	7-11	ApoB-100	ApoC-II, C-III, E
LDL	1.019-1.063	20-25	4-6	34-35	22-26	6-15	ApoB-100	
HDL	1.063-1.210	8-13	7	10-20	55	5	ApoA-I	ApoA-II, C-II, E, M
Lp(a)	1.006-1.125	25-30	4-8	35-46	17-24	6-9	Apo(a)	ApoB-100

Apo - apolipoprotein; HDL - high-density lipoprotein; IDL - intermediate-density lipoprotein; LDL - low-density lipoprotein; Lp(a) - lipoprotein(a); PLs - phospholipids; TGs - triglycerides; VLDL - very low-density lipoprotein. Adapted from Ref. [5].

Table 1.
Physical and chemical characteristics of human plasma lipoproteins.

Apolipoprotein	Location	Origin	Function
Apo A-I	HDL	Liver, intestine	Major component of HDL, activates LCAT
Apo A-II	HDL	Liver	Component of HDL
Apo B-48	Chylomicrons	Intestine	Major component of chylomicron, synthesized in the intestine
Apo B-100	VLDL, IDL, LDL, Lp(a)	Liver	LDL receptor ligand
Apo C-II	Chylomicrons, VLDL, HDL	Liver	co-factor for LPL, stimulates triglyceride hydrolysis
Apo C-III	Chylomicrons, VLDL, HDL	Liver	Inhibits LPL
Apo E	Chylomicrons, remnants, VLDL, HDL	Liver, intestine	LDL receptor ligand
Apo(a)	Lp(a)	Liver	Component of Lp(a), links to LDL, inhibits fibrinolysis

Apo - apolipoprotein; HDL - high-density lipoprotein; IDL - intermediate-density lipoprotein; LCAT - lecithin-cholesterol acyltransferase; LDL - low-density lipoprotein; LPL - lipoprotein lipase; Lp(a) - lipoprotein (a); VLDL - very-low-density lipoprotein.

Table 2.
Major apolipoprotein characteristics.

3.1 Chylomicrons

Chylomicrons are the largest particles in the lipoprotein family with the highest lipid to protein ratio. These triglyceride-rich particles contain apolipoproteins A-I, A-II, A-IV, A-V, B-48, C-II, C-III, and E, nevertheless, apo B-48 is the core structural protein and each chylomicron particle contains one Apo B-48 molecule [6]. The size of chylomicrons varies depending on the amount of fat ingested. A meal high in fat results in the formation of large chylomicron particles due to the increased quantity

of TG being transported whereas in the fasting state the chylomicron particles are smaller since they are carrying decreased amount of TG.

3.2 Chylomicron remnants

The removal of TG from chylomicrons by peripheral tissues results in smaller particles called chylomicron remnants. Compared to chylomicrons these particles are enriched in cholesterol and are pro-atherogenic [7].

3.3 Very low-density lipoproteins (VLDL)

Very low-density lipoproteins are also triglyceride-rich particles, however, they are smaller than chylomicrons and contain relatively less TG but more cholesterol and protein. Similar to chylomicrons the size of the VLDL particles vary depending on the amount of TG carried in the particle. Hence, when TG production in the liver is increased, the secreted VLDL particles are large. VLDL particles contain apolipoproteins B-100, C-I, C-II, C-III, and E. Apo B-100 is the core structural protein and each VLDL particle contains one Apo B-100 molecule [8].

3.4 Intermediate-density lipoproteins (IDL; VLDL remnants)

The removal of TG from VLDL by peripheral tissue (muscle and adipose tissue) results in the formation of IDL particles which are enriched in cholesterol. These particles contain apolipoprotein B-100 and E and are pro-atherogenic [8].

3.5 Low-density lipoproteins (LDL)

Low density lipoproteins are derived from VLDL and IDL particles by the lipoprotein lipase-mediated intravascular removal of TGs and are further enriched in cholesterol. Therefore, the LDL inner core is predominately composed of cholesterol esters. LDL particles are the primary transport mechanism for the delivery of cholesterol to peripheral tissues, accounting for the majority of circulating cholesterol in humans. Apo B-100 is the predominant structural protein and each LDL particle contains one Apo B-100 molecule [3]. LDL comprise a range of particles differing in size and density. Small dense LDL (sdLDL) particles are considered to be more atherogenic than larger LDL subfractions [9]. A growing body of evidence suggests that sdLDL particles have a decreased affinity for the LDL receptor resulting in a prolonged retention time in the circulation. Longer circulation times lead to multiple atherogenic modifications of sdLDL particles, further increasing its atherogenicity. Moreover, sdLDL particles bind more avidly to intraarterial proteoglycans and are characterized by the enhanced ability to enter the arterial wall. Finally, sdLDL particles are more susceptible to oxidation, which could result in an enhanced uptake by macrophages [10].

The predominance of sdLDL has been associated with hypertriglyceridemia, low HDL and high-hepatic lipase activity. This lipid phenotype was found to be present across the broad spectrum of metabolic disorders including obesity, metabolic syndrome, type 2 diabetes and is considered as a risk factor of coronary heart disease.

3.6 High-density lipoproteins (HDL)

High density lipoproteins are the smallest particles in the lipoprotein family composed of a relatively high proportion of protein thus having the lowest lipid to protein ratio. Their core is mainly composed of cholesterol esters. HDL particles

contain apolipoproteins A-I, A-II, A-IV, C-I, C-II, C-III, and E. Apo A-I is the core structural protein and each HDL particle may contain multiple Apo A-I molecules. The main physiological role of HDL is in the transport of cholesterol from peripheral tissues to the liver, which is one possible mechanism to explain their ability to inhibit atherosclerosis [11]. In addition, HDL particles have anti-oxidant, anti-inflammatory, anti-thrombotic, and anti-apoptotic properties, which may also contribute to their anti-atherogenic potential. HDL comprise a range of particles varying in size, density and apolipoprotein composition.

3.7 Lipoprotein(a) [Lp(a)]

Lipoprotein(a) consists of an LDL particle and the specific apolipoprotein(a), which is attached via a single disulfide bond to the Apo B-100. Lp(a) contain Apo(a) and Apo B-100 in a 1:1 molar ratio. The structure of apolipoprotein(a) is similar to plasminogen and tissue plasminogen activator (tPA) containing multiple kringle repeats. Due to a variable number of kringle repeats, each of which consists of 114 amino acids, the molecular weight of apo(a) isoforms can range from 250,000 to 800,000 [12]. The production rate of Lp(a) is predominantly genetically determined resulting in highly variable Lp(a) plasma concentration ranging from undetectable to more than 200 mg/dl. There is a general inverse correlation between the Lp(a) concentration in plasma and the size of the apo(a) isoform. Individuals with low molecular weight Apo (a) tend to have higher levels while individuals with high molecular weight Apo(a) isoforms tend to have lower levels of Lp(a). It is hypothesized that the larger the isoform, the more Apo(a) precursor protein accumulates intracellularly in the endoplasmic reticulum and consequently the liver is less efficient in secreting high molecular weight Apo(a) [13]. The mechanism of Lp (a) clearance is still not fully elucidated but does not seem to include LDL receptors. As kidney disease is associated with an increase in Lp (a) levels, the kidney appears to have an important role in Lp (a) clearance. Elevated plasma Lp(a) levels are associated with an increased risk of atherosclerosis. There are several proposed mechanisms to explain a proatherogenic role of Lp(a). As the structure of Apo(a) is similar to plasminogen and tPA it competes with plasminogen for its binding site, leading to reduced fibrinolysis. Moreover, Lp(a) stimulates the secretion of PAI-1, which results in enhanced thrombogenesis. Also, Lp(a) particles are preferential carriers of atherogenic pro-inflammatory oxidized phospholipids in human plasma that attracts inflammatory cells to vessel walls and stimulate smooth muscle cell proliferation [14]. However, statin therapy as well as other therapies that accelerate LDL clearance and decrease LDL levels do not decrease Lp(a) levels [15].

4. The role of lipids and lipoproteins in atherogenesis

Consistent evidence from epidemiologic and clinical studies, supports the key role of the apoB containing lipoproteins in atherogenesis. All ApoB-containing lipoproteins with size less than 70 nm can cross the endothelial barrier, particularly in the presence of endothelial dysfunction [16]. Uptake and accumulation of apoB-containing lipoproteins in the arterial wall is a critical initiating event in the development of atherosclerosis. Upon entry, apoB-containing lipoproteins are modified and oxidized into proinflammatory particles, which provoke the activation of the innate immune system within the arterial intima. The endothelial cells secrete adhesion molecules, and the smooth muscle cells (SMCs) secrete chemokines, which together attract monocytes and other immune cells into the arterial wall. When monocytes enter the subendothelial space, they transform into macrophages.

Macrophage inflammation leads to enhanced oxidative stress and cytokine secretion, further promoting apoB-containing lipoproteins oxidation, endothelial cell activation, proliferation of SMCs and monocyte recruitment [17]. Uptake of the apoB containing particles by macrophages promotes foam cell formation which accumulate in early atherosclerotic lesions known as fatty streaks [18]. Fatty streaks are not clinically significant, but they are the precursors of more advanced lesions characterized by the accumulation of lipid-rich necrotic debris and SMCs. With the secretion of fibrous elements by the SMCs, forming a fibrous cap over the lipid-rich necrotic cores, atherosclerotic fibrous plaques develop. Continued exposure to apoB-containing lipoproteins results in additional particles being retained over time in the arterial wall, and to the growth and progression of atherosclerotic plaques [19]. A person's total atherosclerotic plaque burden is determined by the concentration of circulating LDL and other apoB-containing lipoproteins, and by the cumulative exposure to these particles. In general, people with higher concentrations of plasma apoB-containing lipoproteins will retain more particles and accumulate lipids faster, resulting in more rapid growth and the progression of atherosclerotic plaques ultimately leading to the reduced vascular lumen and clinically significant ischemia. Plaques can become increasingly complex, with calcification, ulceration at the luminal surface, and hemorrhage within the arterial wall from small fragile vessels growing into the lesion from the media. Eventually, changes in the composition of the plaque reach a critical point at which disruption of a plaque can result, with the formation of an overlying thrombus that acutely obstructs blood flow. Atherosclerotic plaque formation is greatest at the branching points of major vessels and in areas of turbulent flow [17]. Depending on the location, atherosclerosis may lead to a variety of conditions, such as coronary heart disease, cerebrovascular and peripheral artery disease.

Epidemiological studies have consistently shown that HDL-C levels are inversely related to atherosclerotic cardiovascular events [20, 21]. HDL-C has been traditionally considered as „good "cholesterol having a protective role against atherosclerosis. The proposed mechanism underlying its protective effect is the role in the removal of excess cholesterol from peripheral tissues. Besides, it has been considered that HDL prevents lipoprotein oxidation and removes oxidized lipids from LDL due to its anti-inflammatory and anti-oxidant properties. However, the protective role has been seriously challenged by the evidence from recent clinical trials aimed at raising HDL-C that failed to reduce cardiovascular events. Modern genome-wide and Mendelian randomization studies have failed to show a causal link between total HDL-C concentration and CAD, which might be related to the fact that HDL comprise a range of particles differing in size and density [22, 23]. It has been shown that the concentration of large HDL particles is inversely associated with CVD while that of small HDL particles is positively associated with CVD.

5. Clinical classification of dyslipidemias

Dyslipidemia may present as a single disorder affecting only one specific type of lipid, such as pure or isolated hypertriglyceridemia or hypercholesterolemia or may represent as a combination of lipid abnormalities, such as mixed or combined dyslipidemias. Lipid disorders were traditionally categorized by patterns of elevation in lipids and lipoproteins into six phenotypes according to the Fredrickson classification (**Table 3**). A more practical approach is to classify dyslipidemias as primary or secondary. Primary dyslipidemias are genetic disorders caused by single or multiple gene mutations that result in either overproduction or defective clearance of LDL and TG or excessive clearance of HDL. The understanding of the

Phenotype	Elevated Lipoprotein(s)	Elevated Lipids
I	Chylomicrons	TG
IIa	LDL	Cholesterol
IIb	LDL and VLDL	TG and cholesterol
III	VLDL and chylomicron remnants	TG and cholesterol
IV	VLDL	TG
V	Chylomicrons and VLDL	TG and cholesterol

LDL - low-density lipoprotein; TG - triglycerides; VLDL - very-low-density lipoprotein.

Table 3.
Fredrickson classification of dyslipidemias.

genetic and biochemical basis of these disorders has revealed a large and diverse group of diseases, many of which have similar clinical expressions (**Table 4**) [24]. The names of many primary dyslipidemias reflect an old nomenclature in which lipoproteins were differentiated by how they separated into alpha (HDL) and beta (LDL) bands on electrophoretic gels. Individuals with primary dyslipidemias are at higher risk of developing complications, such as atherosclerotic cardiovascular disease, at a younger age. Patients may also present with acute pancreatitis and deposition of cholesterol in the skin and tendons (xanthomas), eyelids (xanthelasma), and corneas (arcus corneae).

Familial hypercholesterolemia (FH) is one of the most common monogenic lipid disorders associated with premature CVD due to significantly elevated plasma levels of LDL-C. FH is caused by loss-of-function mutations in the LDL receptor or apoB genes, or a gain-of-function mutation in the PCSK9 gene [25]. There are two main types of FH; homozygous (HoFH) and heterozygous (HeFH). The prevalence of HeFH in the population is estimated to be 1/200-250, making it the most common genetically transmitted disease [26, 27]. If left untreated, men and women with HeFH typically develop early coronary artery disease (CAD) before the ages of 55 and 60 years respectively. However, early diagnosis and appropriate treatment can dramatically reduce the risk for CAD. HoFH is a rare and life-threatening disease with a prevalence estimated to be 1/160,000-320,000. Patients present with extensive xanthomas, premature and progressive CVD, and total cholesterol level exceeds 13 mmol/L. Most patients die before 30 years of age [28].

Secondary dyslipidemias are caused by lifestyle factors or medical conditions that interfere with blood lipid levels [29, 30]. The most important cause is a sedentary lifestyle with excessive dietary intake of total calories, saturated fats, cholesterol and trans fats. Some diseases that are associated with a higher risk of dyslipidemia are diabetes mellitus, cholestatic liver disease, chronic kidney disease, nephrotic syndrome, hypothyroidism and obesity [31, 32].

Diabetes is an especially important secondary cause of dyslipidemia characterized by an atherogenic combination of high TGs, high sdLDL particles and low HDL [33]. Patients with type 2 diabetes are particularly at risk [34]. It has been shown that the lipoprotein abnormalities are related to the severity of the insulin resistance and the degree of visceral adiposity. Poor glycemic control and inflammation of visceral adipose tissue increase the concentration of circulating free fatty acids (FFAs), leading to increased VLDL production in the liver. TG-rich VLDL then transfers TG and cholesterol to LDL and HDL, promoting formation of TG-rich, sdLDL and clearance of TG-rich HDL. Diabetic dyslipidemia is additionally promoted by unhealthy diet and physical inactivity. Other factors that increase the risk of dyslipidemias are smoking, alcohol overuse and certain medications such as thiazide

Disorder	Genetic Defect	Inheritance	Clinical Features
Apo C-II deficiency	Apo C-II (causing functional LPL deficiency)	Recessive	• metabolic syndrome (often present) • pancreatitis (in some adults) • TG: > 8.5 mmol/L
Cerebrotendinous xanthomatosis	Hepatic mitochondrial 27-hydroxylase defect accumulation of cholestanol due to the blockage of bile acid synthesis and conversion of cholesterol to cholestanol	Recessive	• cataracts • premature CAD • neuropathy • ataxia
Cholesteryl ester storage disease and Wolman disease	Lysosomal acid lipase deficiency	Recessive	• premature CAD • accumulation of cholesteryl esters and TG in lysosomes in the liver, spleen, and lymph nodes • cirrhosis
Familial apo AI deficiency/mutations	Apo AI	Unknown	• corneal opacities, xanthomas, premature CAD (in some people) • HDL: 0.39–0.78 mmol/L
Familial combined hyperlipidemia	Unknown, possibly multiple defects and mechanisms	Dominant	• premature CAD, responsible for about 15% of MIs in people < 60 years • Apo B: Disproportionately elevated • TC: 6.5–13.0 mmol/L • TG: 2.8–8.5 mmol/L
Familial defective apo B-100	Apo B (LDL receptor–binding region defect) Diminished LDL clearance	Dominant	• xanthomas, arcus corneae, premature CAD • TC: 6.5–13 mmol/L
Familial dysbetalipoproteinemia	Apo E (usually e2/e2 homozygotes) Diminished chylomicron and VLDL clearance	Recessive or dominant	• xanthomas (especially tuberous and palmar), yellow palmar creases, premature CAD • TC: 6.5–13.0 mmol/L • TG: 2.8–5.6 mmol/L

Disorder	Genetic Defect	Inheritance	Clinical Features
Familial HDL deficiency	ABCA1 gene	Dominant	• premature CAD
Familial hypercholesterolemia	Loss-of-function mutations in the LDL receptor or apoB genes, or a gain-of-function mutation in the PCSK9 Diminished LDL clearance	Codominant	Heterozygotes: • tendon xanthomas, arcus corneae, premature CAD (ages 30–50), responsible for about 5% of MIs in people < 60 years • TC: 6.5–13 mmol/L Homozygotes: • planar and tendon xanthomas and tuberous xanthomas, premature CAD (before age 18) • TC > 13 mmol/L
Familial hypertriglyceridemia	Unknown, possibly multiple defects and mechanisms	Dominant	• Usually no symptoms or findings; occasionally hyperuricemia, sometimes early atherosclerosis • TG: 2.3–5.6 mmol/L, possibly higher depending on diet and alcohol use
Familial LCAT deficiency	LCAT gene	Recessive	• Corneal opacities, anemia, chronic kidney disease • HDL: < 0.26 mmol/L
Fisheye disease (partial LCAT deficiency)	LCAT gene	Recessive	• Corneal opacities • HDL: < 0.26 mmol/L
Hepatic lipase deficiency	Hepatic lipase	Recessive	• premature CAD • TC: 6.5–39 mmol/L • TG: 4.5–93 mmol/L • HDL: variable

Disorder	Genetic Defect	Inheritance	Clinical Features
LPL deficiency	Endothelial LPL defect Diminished chylomicron clearance	Recessive	• failure to thrive (in infants), eruptive xanthomas, hepatosplenomegaly, pancreatitis • TG: > 8.5 mmol/L
PCSK9 gain of function mutations	Increased degradation of LDL receptors	Dominat	• similar to familial hypercholesterolemia
Polygenic hypercholesterolemia	Unknown, possibly multiple defects and mechanisms	Variable	• premature CAD • TC: 6.5–9.0 mmol/L
Primary hypoalphalipoproteine-mia (familial or nonfamilial)	Unknown, possibly apo A-I, C-III, or A-IV	Dominat	• premature CAD • HDL: 0.39–0.91 mmol/L
Sitosterolemia	ABCG5 and ABCG8 genes	Recessive	• tendon xanthomas, premature CAD
Tangier disease	ABCA1 gene	Recessive	• premature CAD (in some people), peripheral neuropathy, hemolytic anemia, corneal opacities, hepatosplenomegaly, orange tonsils • HDL: < 0.13 mmol/L

ABCA1 - ATP-binding cassette transporter A1; ABCG5 and 8 - ATP-binding cassette subfamily G members 5 and 8; apo - apoprotein; CAD - coronary artery disease; HDL - high-density lipoprotein; LCAT - lecithin-cholesterol acyltransferase; LDL - low-density lipoprotein; LPL - lipoprotein lipase; MI - myocardial infarction; PCSK9 - proprotein convertase subtilisin-like/kexin type 9; TC - total cholesterol; TG - triglyceride; VLDL - very-low-density lipoprotein.
Adapted from Ref. [23].

Table 4.
Primary dyslipidemias.

Medical conditions	**Lipid abnormalities**
Diabetes mellitus, metabolic syndrome	↑ sdLDL-C, ↓ HDL-C, ↑ TG
Cholestatic liver disease	↑ TC ↑ LDL-C
Nephrotic syndrome	↑ TC, ↑ LDL-C
Chronic kidney disease	↑ LDL-C, ↓ HDL-C, ↑ TG
Hypothyroidism	↑ LDL-C, ↑ TG
Obesity	↑ TC, ↑ LDL-C, ↓ HDL-C, ↑ TG
Cigarette smoking	↓ HDL-C
Excessive alcohol consumption	↑ TG
Medications	
Diuretics, cyclosporine, glucocorticoids, amiodarone	↑ LDL-C
Oral estrogens, glucocorticoids, protease inhibitors, sirolimus, beta blockers, thiazides, anabolic steroids	↑ TG

HDL-C - high density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol; sdLDL-C -small dense low density lipoprotein cholesterol; TC-total cholesterol; TG - triglycerides.

Table 5.
Secondary causes of dyslipidemia.

diuretics, beta blockers, oral contraceptives, atypical antipsychotics, antiretroviral agents, corticosteroids, tacrolimus, and cyclosporine. Secondary causes of dyslipidemia and their major lipid abnormalities are shown in **Table 5**.

6. Treatment strategies

6.1 Impact of diet and lifestyle modifications on lipid levels

Consistent evidence from epidemiological studies indicates that saturated fatty acids (SFAs) and trans unsaturated fatty acids are the dietary factors with the greatest elevating impact on LDL-C levels [5]. Therefore, current dietary guidelines uniformly recommend reducing intakes of saturated and trans fatty acids with replacement by increasing intake of mono- and polyunsaturated fatty acids [35]. Moreover, recommended food choices to lower LDL-C and improve the overall lipoprotein profile include higher consumption of non-starchy vegetables, fruit, legumes, nuts, fish, vegetable oils, yoghurt, and wholegrains, along with a lower intake of red and processed meats, foods higher in refined carbohydrates, and salt [36, 37]. Dietary patterns that may have a role in the prevention and management of dyslipidemia are the Mediterranean diet and the DASH diet [38, 39]. Excessive body weight loss exhibits the LDL-C decreasing effect, but the magnitude of the effect is small. In people with obesity, a decrease in LDL-C concentration of 0.2 mmol/L is observed for every 10 kg of weight reduction [40, 41]. Regular physical exercise results in even smaller reduction of LDL-C levels [42, 43]. Overall, through dietary changes and weight loss, LDL-C can be lowered by approximately 10–15% [44].

6.2 Drugs for treatment of dyslipidemias

Statin treatment, targeting LDL cholesterol reduction, remains the cornerstone of dyslipidemia management. There is a clear linear relationship between the degree of LDL-cholesterol lowering achieved with statins and CV benefits, pointing out

that a reduction of 1 mmol/L of LDL-C is associated with a 20–25% reduction in the relative risk of major CV events including cardiovascular mortality, non-fatal myocardial infarction and non-fatal stroke [45]. Statins reduce the biosynthesis of cholesterol in the liver by competitively inhibiting the enzyme hydroxymethylglutaryl CoA (HMG-CoA) reductase, the rate-limiting step in the production of cholesterol. The reduction in intracellular cholesterol promotes up-regulation of LDL receptor (LDLR) at the surface of the hepatocytes, which in turn results in increased hepatic uptake of LDL from the blood, thereby lowering plasma concentrations of LDL- and other ApoB-containing lipoprotein particles. The degree of LDL-C reduction is dose-dependent and varies between the different statins. A high intensity statin, on average, reduces LDL-C by >50%, while, moderate-intensity therapy is defined as the dose expected to reduce LDL-C by 30-50% [35]. Statins should be initiated with the highest tolerated dose to reach the LDL-C goal determined by the individual's risk category. There are abundant data supporting the concept of 'the lower LDL-C, the better' in the primary and secondary cardiovascular disease prevention. Statins are generally safe and well tolerated apart from myalgia which is the most commonly reported statin adverse effect, although its frequency is higher in everyday clinical practice than in RCTs [46]. However, due to low adherence to statin therapy or statin intolerance, many patients do not reach LDL-C target levels. Because the LDL-C targets suggested in guidelines, currently <1.4 mmol/L in patients with very-high CV risk, < 1.8 in patients with high CV risk and < 2.6 mmol/L in those with moderate CV risk respectively, are often not achieved, additional and more aggressive LDL-C lowering therapies are needed [35].

Ezetimibe inhibits dietary and biliary cholesterol absorption by interacting with the Niemann-Pick C1-Like 1 protein (NPC1L1), thereby lowering the amount of cholesterol delivered to the liver. In response to reduced cholesterol delivery, the liver reacts by upregulating LDL receptor expression, which in turn leads to increased clearance of LDL from the blood. A large clinical trial evaluating the addition of ezetimibe to statins in patients with prior acute coronary syndrome found a 24% reduction in LDL-C levels and a 6.4% reduction in the relative risk of CV death, major coronary events, or nonfatal stroke at 7 years [47]. Statin-ezetimibe combination treatment is the first choice for managing elevated LDL-C in very-high-risk patients with high LDL-C unlikely to reach goal with a statin, and in primary prevention familial hypercholesterolaemia patients [48].

A new class of drugs, PCSK9 inhibitors, that targets a proprotein convertase subtilisin/kexin type 9 (PCSK9) is recommended by current guidelines for the secondary prevention of very high-risk individuals not at LDL-C goal despite maximally tolerated statin doses and ezetimibe [35]. This protein regulates plasma concentrations of LDL-C by interacting with LDL receptors on hepatocytes. After binding to an LDL receptor, PCSK9 directs it to lysosomal degradation. Consequently, it inhibits recycling of the receptor to the surface of the hepatocyte and delays catabolism of LDL particles [49]. Currently approved PCSK9 inhibitors are the human monoclonal antibodies, alirocumab and evolocumab. The mechanism of action relates to the reduction of the plasma level of PCSK9, which in turn results in decreased intracellular degradation and increased expression of LDL receptors at the cell surface and therefore in a reduction of circulating LDL-C levels [50]. Co-administration with statin treatment has a sound rationale because statins upregulate PCSK9. In clinical trials, PCSK9 inhibitors either alone or in combination with statins, and/or other lipid-lowering therapies have been shown to significantly reduce LDL-C levels on average by 60%, depending on dose. In contrast to statins, inhibiting PCSK9 with monoclonal antibodies also reduces Lp(a) plasma levels.

An alternative approach targeting PCSK9 consists of RNA interference. Recently, the small interfering RNA (siRNA) molecule inclisiran, which inhibits the intracellular hepatic translation of PCSK9, has been approved in Europe based on a robust clinical development program demonstrating effective and sustained LDL-C reduction of up to 52% in patients with elevated LDL-C despite maximally tolerated statin therapy [51, 52]. With two doses a year, this new lipid lowering strategy is expected to support long-term adherence.

The cholesterol efflux capacity, mainly mediated by HDL-C, from arterial tissues to liver has demonstrated its association with major adverse cardiovascular events [53]. The pharmacological approach that has led to the greatest elevations in HDL-C levels has been direct inhibition of cholesterol ester transfer protein (CETP) by small-molecule inhibitors, which may induce an increase in HDL-C by >100% on a dose-dependent basis. Although CETP inhibitors significantly increased HDL-C levels in trials, they have not displayed benefits on cardiovascular outcomes [22].

Hypertriglyceridemia is a well-described contributor to the residual cardiovascular risk [54]. Statin treatment is recommended as the first drug of choice to reduce CVD risk in high-risk individuals with TG levels >2.3 mmol/L. In high and very high-risk patients with TG levels between 1.5-5.6 mmol/L despite statin treatment, n-3 PUFAs (icosapent ethyl 2x2 g/day) should be considered in combination with a statin. It has been demonstrated that icosapent ethyl, a highly purified and stable eicosapentaenoic acid (EPA), on top of statins was associated with a 25% relative CV risk reduction and a 4.8% absolute risk reduction in major adverse CV events in high-risk individuals [55]. The underlying mechanism how omega-3 fatty acids affect serum lipids and lipoproteins, in particular VLDL concentrations is poorly understood, although it may be related, at least in part, to their ability to interact with peroxisome proliferator-activated receptors (PPARs) and to decreased secretion of ApoB. In primary prevention patients who are at LDL-C goal with TG levels >2.3 mmol/L, fenofibrate may be considered in combination with statins. Fibrates are agonists of PPARs, acting via transcription factors regulating various steps in lipid and lipoprotein metabolism. Consequently, fibrates have good efficacy in lowering fasting and post-prandial TGs and TG-rich lipoprotein remnant particles [56, 57].

Agents that enhance catabolism of TG-rich lipoproteins, such as the antisense oligonucleotide to ApoC-III mRNA, which lead to a concomitant reduction in TGs (>70%) and a marked elevation in HDL-C (>40%) in hypertriglyceridemia, are under development [58].

7. Conclusion

Dyslipidemias largely contribute to global cardiovascular disease burden. Consistent evidence from epidemiological and clinical studies, supports the key role of the circulating LDL-C and other apoB containing lipoproteins in the development of atherosclerosis. Therefore, reducing LDL-C and other ApoB-containing lipoproteins is a core component of lipid management for both the primary prevention of CVD and the secondary prevention of recurrent CV events. A major outstanding challenge is how best to implement use of evidence-based therapies in clinical practice, particularly statins and PCSK9 inhibitors. Understanding the important role that metabolic derangements play in the pathogenesis of atherosclerosis pave the way for stronger implementation of current guidelines for CVD risk assessment and prevention.

Author details

Sanja Klobučar Majanović[1,2], Olga Cvijanović Peloza[3]*, Dijana Detel[4], Gordana Kenđel Jovanović[5], Miro Bakula[6,7], Dario Rahelic[7,8,9], Tea Čaljkušić Mance[10,11], Tamara Šoić Vranić[3] and Sanja Zoričić Cvek[3]

1 Department of Endocrinology, Diabetes and Metabolic Diseases, Clinical Hospital Centre Rijeka, Rijeka, Croatia

2 Department of Internal Medicine, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

3 Department of Anatomy, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

4 Department of Chemistry and Biochemistry, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

5 Department of Health Ecology, Teaching Institute of Public Health of Primorsko-goranska County, Rijeka, Croatia

6 Department of Endocrinology, Diabetes and Metabolic Disorders, Clinical Hospital "Sveti Duh", Zagreb, Croatia

7 School of Medicine, University of Zagreb, Zagreb, Croatia

8 Vuk Vrhovac University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Zagreb, Croatia

9 School of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

10 Department of Ophtalmology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

11 Clinical Hospital Centre Rijeka, Rijeka, Croatia

*Address all correspondence to: olga.cvijanovic@uniri.hr

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Chapter 2

Structure of Lipoproteins and Their Capacity for Lipid Exchange: Relevance for Development of Atherosclerosis and Its Treatment by HDL Therapy

Sarah Waldie, Rita Del Giudice and Marité Cárdenas

Abstract

Atherosclerosis, the largest killer in the western world, arises from build-up of plaques at the artery walls and can result in cardiovascular disease. Low- and high-density lipoproteins are involved in the disease development by depositing and removing lipids to and from macrophages at the artery wall. These processes are complex and not fully understood. Thus, determining the specific roles of the different lipoprotein fractions involved is of fundamental importance for the treatment of the disease. In this chapter, we present the state of the art in lipoprotein structure with focus on the comparison between normolipidemic and hypertriglyceridemic individuals. Then we discuss lipid transfer between lipoproteins and receptor-free cellular membranes. Although these models lack any receptor, key clinical observations are mirrored by these, including increased ability of HDL to remove lipids, in contrast to the ability of LDL to deposit them. Also effects of saturated and unsaturated lipids in the presence and absence of cholesterol are revised. These models can then be used to understand the difference in functionality of lipoproteins from individuals showing different lipid profiles and have the potential to be used also for the development of new HDL therapies.

Keywords: lipoprotein structure, SAXS, lipid exchange, lipid transfer, neutron scattering

1. Introduction

This chapter starts by discussing the main compositional and structural properties of lipoproteins, which are water-soluble, heterogeneous nanoparticles responsible for carrying lipids, cholesterol and triglycerides in the body. The differences in their composition and structure are strictly related to how the different lipoproteins are produced and what their roles are in the body. We then move on to discuss the relationship between how lipoprotein type and lipoprotein subclass relate with the risk to develop atherosclerosis. We review recent evidence that suggests differences in low-density lipoprotein (LDL) overall size, shape and protein layer thicknesses within small dense LDL subfractions of normolipidemic and hyper triglyceridemic

individuals. This is of importance since structural differences across a certain lipoprotein class or subclass are not taken into account in clinical studies and this might explain controversies in the role of, for example, small dense LDL in the development of atherosclerosis. We then move on to discuss how lipoprotein capacity for lipid transfer and lipid exchange has been studied along the years and focus on recent results that quantify these abilities using simplistic model membranes lacking specific receptors. Despite the simplicity of these model systems, the results mirror those obtained for cholesterol efflux and in clinicals studies. Finally, we discuss advances in plaque remodelling therapies based on the engineering of nanoparticles mimicking nascent high-density lipoprotein (HDL) particles with focus on the challenges for the formulation of therapies that are effective in the clinics.

2. Lipoproteins are nanoparticles, made of lipids and apolipoproteins

2.1 Lipoprotein metabolism

Lipoproteins are the carriers of fat in the body. Plasma lipoproteins are secreted mostly by the liver and the intestine, whereas lipoproteins carrying lipids in the central nervous system are secreted mainly by the glial cells [1]. In **Figure 1**, the two ways by which plasma lipoproteins are formed are summarised. Chylomicrons, the largest and the least dense lipoprotein type, are produced in the intestine after a meal. Their size varies and depends on the amount of fat consumed during a meal. For instance, during fasting, chylomicrons are small and contain low amounts of triglycerides whereas a high-fat meal will result in the formation of larger particles,

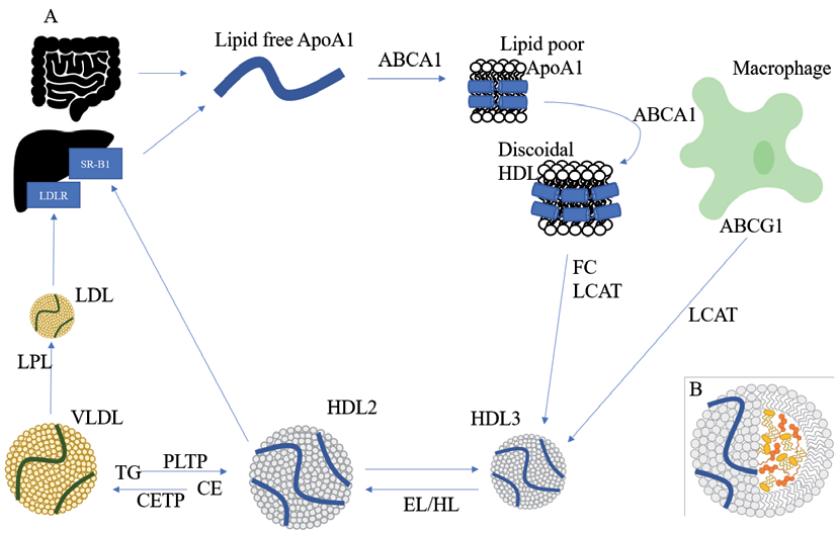

Figure 1.
(A) Lipoprotein metabolism: Lipid-free ApoA1 is produced by both the intestine and the liver. It gains phospholipids and cholesterol via *the ATP binding cassette A1 (ABCA1) to form lipid-poor ApoA1; it gains further lipids from peripheral tissues to form nascent discoidal HDL, which obtains free cholesterol (FC) from macrophages* via *interaction with ABCA1 and ABCG1 transporters. The FC is esterified by lecithin-cholesterol acyltransferase (LCAT) to form mature spherical HDL. Mature HDL can interact with the scavenger receptor class B type-1 (SR-B1) in the liver resulting in the exchange of unesterified cholesterol in both directions. Cholesteryl esters (CE) are transferred to VLDL* via *the cholesteryl ester transfer protein (CETP) for eventual uptake by the LDLR in the liver. The progression from VLDL to LDL occurs* via *hydrolysis by lipoprotein lipases (LPL). Phospholipids and triglycerides (TG) are transferred to HDL from VLDL* via *the phospholipid transfer protein (PLTP) resulting in HDL remodelling. Hepatic and endothelial lipases (HL/EL) also promote HDL remodelling. Insert: B. lipoprotein structure with a core of cholesterol esters and triglycerides.*

rich in triglycerides [2]. They undergo hydrolysis of triglycerides resulting in chylomicron remnants which are cleared *via* the liver [3]. Then, very low-density lipoproteins (VLDL) are produced in the liver. VLDL are the next least dense lipoprotein type, rich in triglycerides and their size depends on the production of triglycerides in the liver: the higher the production, the larger the secreted VLDL. These particles are hydrolysed in muscle and adipose tissues by lipoprotein lipases (LPL) resulting in the removal of the triglycerides and the formation of intermediate density lipoprotein (IDL), which are lipoprotein particles rich in cholesterol [2]. Further hydrolysis *via* LPL occurs and results in the formation of LDL, which is even richer in cholesterol and accounts for the principal carrier of cholesterol in circulation.

HDL formation and metabolism, on the other hand, starts with the production of apolipoprotein A1 (ApoA1) in the intestine and liver [4]. ApoA1 initial lipidation starts with the addition of both phospholipids and cholesterol from peripheral tissues *via* its interaction with the ATP-binding cassette A1 (ABCA1) transporter. Further lipid uptake mediated by ABCA1 and ABCG1 transporters leads to the formation of the "nascent", discoidal HDL. Free cholesterol is then esterified by lecithin-cholesterol acyltransferase (LCAT), resulting in the formation of mature, spherical HDL with a core full of cholesterol esters [4]. Both nascent and mature HDL can interact with the scavenger receptor class B type-1 (SR-B1) in the liver and undergo transfer of cholesterol esters towards SR-B1 and exchange of unesterified cholesterol in both directions [5]. The transfer of cholesterol esters also occurs *via* the cholesteryl ester transfer protein (CETP) to VLDL and LDL for eventual uptake by the LDL receptor (LDLR) in the liver [6]. The transfer of phospholipids and triglycerides from VLDL to HDL is facilitated by phospholipid transfer protein (PLTP), resulting in HDL remodelling. The hydrolysis of HDL phospholipids and triglycerides *via* hepatic and endothelial lipases (HL and EL) also results in HDL remodelling [7].

2.2 Lipoprotein composition

Lipoproteins are very heterogeneous in composition and differ in terms of their proportions of constituent proteins, cholesterol/cholesteryl esters, triglycerides and phospholipids (**Table 1**). The proteins responsible for the stability of the lipoprotein structure and their function in lipid transport and metabolism are known as apolipoproteins. Apolipoproteins vary in their size and overall structure, but they share a structure rich in amphipathic alpha helices, which are prone to interact with lipids and fats in aqueous environments such as plasma. In general, the larger the particle the higher the content ratio between lipids to proteins, which makes them less dense. Lipoproteins differ also in the main apolipoprotein they present. Apolipoprotein B100 (ApoB100) [9] is present only in VLDL, IDL

	VLDL	LDL	HDL
Diameter/ nm	30–80	18–25	5–12
Cholesterol/% (w/w)	~6	~7	~4
Phospholipids/% (w/w)	~17	~21	~29
Triglycerides/% (w/w)	~55	~6	~4
Protein/% (w/w)	~8	~20	~50

Table 1.
Biochemical composition and size of major lipoprotein types [8].

and LDL, since they are all formed from chylomicrons. ApoB100 is one of the largest proteins known (~4500 residues) and therefore is irreversibly bound to these particles. The rest of the apolipoproteins are reversible and exchange between the different lipoprotein types. For example, Apolipoprotein C (ApoC) and Apolipoprotein E (ApoE) are commonly found in VLDL and LDL [10]. The main protein present in HDL is ApoA1, contributing to about 70% of total protein content in all HDL [10]. The second most abundant is Apolipoprotein A2 (ApoA2) [10] followed by a combination of various other proteins including ApoC, E and Apolipoprotein J. While almost all HDL contain ApoA1 [11], the remaining apolipoproteins vary across different HDL types and subclasses [10].

2.3 Lipoprotein classification

As discussed previously, lipoprotein types vary drastically in their biochemical composition, which determines their size and density (**Table 1**). To further increase the degree of complexity, there are more subdivisions to these main categories. For example, using ultra-centrifugation, LDL can be subdivided in six subfractions which range from the large buoyant LDL (lbLDL) subfractions to the small dense LDL (sdLDL) subfractions [12, 13]. As their names suggest, these subfractions differ in size and density. For HDL, there are generally five distinct subpopulations with slight variations in composition, but most notably differences in size and density. HDL was first described by differences in density according to ultra-centrifugation techniques categorising HDL into two distinct groups [14]: i) HDL2, which is lower in density due to a higher lipid content (1.063–1.125 g mL^{-1}), and ii) HDL3, which is slightly higher in density owing to its higher protein content (1.125–1.21 g mL^{-1}). These groups can be further categorised by their size, using polyacrylamide gradient gel electrophoresis (GGE) resulting in five additional subclasses ranging in size from 7.2–12.0 nm in diameter [15]. By means of GGE, HDL particles can also be classified based on their surface charge into particles that migrate to either pre- β - or α-positions. Pre- β-position corresponds to lipid-free ApoA1, lipid-poor ApoA1, and most discoidal HDLs, whereas spherical, mature HDL migrate to α -position [16].

2.4 Lipoprotein structure

The structure of lipoproteins was studied already in the late 1970s by small angle X-ray scattering (SAXS) [13, 17–19] and in the early 1980s by small angle neutron scattering (SANS) [20]. It was then proposed their core-shell structure today widely accepted: lipoproteins are nanoparticles that consist of a core of cholesterol esters and triglycerides, with an outer monolayer of lipids and cholesterol, all encased by apolipoproteins. For the cholesterol richer LDL, a gel-liquid phase transition occurs below body temperature, T_m. In the early SAXS-based structure, the core was thought to organise in cholesteryl rich concentric layers maintaining a spherical structure [13] below T_m. More recently, cryogenic transmission electron microscopy (cryo-EM) was used to demonstrate that LDL particles are ellipsoidal having a stack of parallel cholesteryl ester layers below T_m [21–23]. The SAXS model for LDL was then revised in 2017 to account for LDL super-ellipsoid shape and planar lamellar ordered cholesteryl ester layer packing in the core below T_m (**Figure 2A**), and spherical LDL with the melted core above T_m [25] (**Figure 2B**). In contrast to cryo-EM, SAXS allows the probing of a large number of LDL particles in a relatively short time and in a physiological-like environment. Therefore, larger and systematic studies can be done providing a range of structural detail that is unprecedented: not only the size but also the shape of the overall particle can be determined, as well as the inner structure of the core and the outer protein shell.

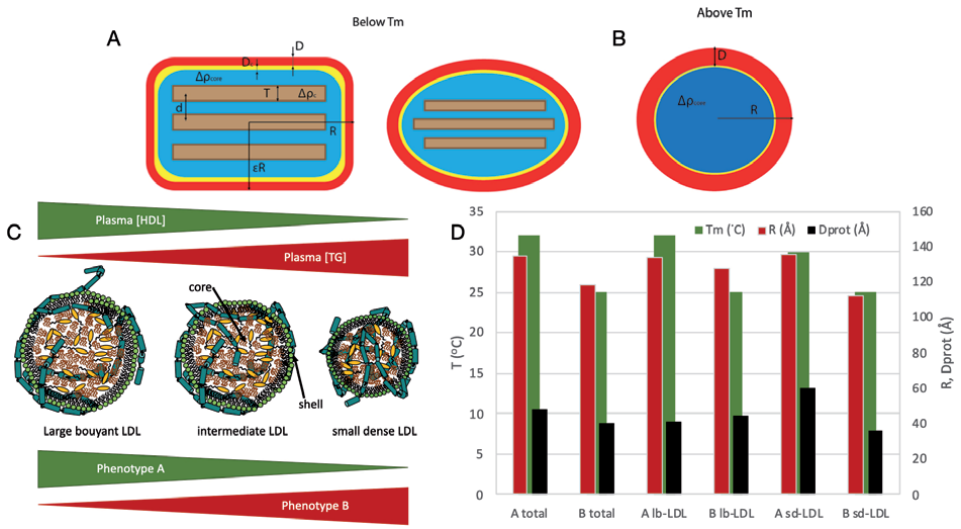

Figure 2.
The updated LDL model based on SAXS data below (A) and above T_m *(B) describes a superellipsoid of revolution or a sphere with core-shell structure, respectively. LDL phenotype A described a LDL sample enriched with lbLDL while phenotype B describes a LDL fraction enriched with sdLDL. Phenotype B is linked to a higher risk to develop atherosclerosis (C) Some structural parameters for total LDL fractions as well as LDL subfractions lbLDL and sdLDl for subject A presenting normal lipid serum values and subject B presenting high triglyceride serum values (D) Data in D is taken from Jakubauskas* et al *2020 [24].*

3. Lipoproteins and atherosclerosis

Atherosclerosis is a chronic inflammatory disease considered to be the largest killer in western countries [26]. It is characterised by the plaque build-up in artery walls and can lead to cardiovascular diseases (CVD), giving rise to ischemic heart disease and strokes [27]. It is accepted that structural and compositional differences between HDL and LDL play a large role in their contributions to atherosclerosis [28]. The plaque build-up originates from LDL deposition into the artery walls [29], which are then oxidised and cause the upregulation of adhesion molecules on the surface of the endothelium of the arteries and the recruitment of monocytes to the forming lesion [30]. These then differentiate in macrophages which express scavenger receptors on their surface that mediate the uptake of even more LDL. This leads to the transformation of macrophages into foam cells, and the formation in the arteries of plaques that consist of apoptotic and necrotic macrophages, cholesterol crystals and other extracellular components [31]. Upon the rupture of these plaques, thrombus material enters the blood stream and can lead to heart attacks or other CVD related phenomena. As opposite to the role of LDL in atherosclerosis development, HDL have been instead shown to play a preventative role by a process known as reverse cholesterol transport (RCT) [32]. By this process, cholesterol is removed from the lipid-filled foam cells at the artery wall and deposited in the liver where it is cleared from the body [33–35]. The cholesterol removal occurs *via* efflux by various means, including passive aqueous diffusion or active, receptor/transporter mediated transfer [36]. Additionally, the presence of HDL has been shown to prevent the oxidation of the LDL and therefore helps prevent the development to atherosclerosis [37]. While both lipoprotein types are characterised by a lipid binding activity, their abilities to exchange lipids and to transport cholesterol differ drastically. Indeed, LDL mainly deposit lipids and cholesterol to artery walls, whereas the main role of HDL in the blood is to catalyse cholesterol efflux thereby removing excess cholesterol from macrophages

trapped at artery walls [28]. Therefore, HDL and LDL are commonly known as 'good' and 'bad' cholesterol respectively.

There are several controversies regarding the role of HDL and LDL in the development of atherosclerosis. On one hand, increased levels of HDL are correlated to reduced atherosclerotic risk due to increased RCT efficiency [35] and, in some other cases, shown to have a neutral [38] or even negative correlation [39] to the prevention of atherosclerotic development. On the other hand, high LDL levels are related to high risk for development of atherosclerosis even though the total cholesterol LDL concentration often fails as a CVD biomarker [40]. This could be due to the pro-atherogenic effect of LDL [41] being counteracted by the anti-atherogenic effect of HDL [42]. Indeed, the ratio between LDL and HDL has been suggested as a better biomarker than their individual serum levels [40].

3.1 Different atherogenic capacity of lipoprotein subclasses

The LDL subclass distribution varies significantly within the population with two main LDL phenotypes [43]: the healthy one (phenotype A), enriched with lbLDL subfractions, and the unhealthy one (phenotype B), enriched with sdLDL subfractions [44] (**Figure 2C**). LDL size is an alleged marker for CVD risk since there is a positive correlation between LDL size and HDL concentration, and a negative correlation between LDL size and triglyceride concentration [43]. However, it is unclear whether it is the LDL concentration or their particle size which is the key parameter to predict CVD risk. Interestingly, there is some genetic, age and sex related predisposition for sdLDL. For example, a low-fat diet can contribute to a phenotype B in individuals with a genetic predisposition for sdLDL [45].

Recently, sdLDL fractions of normal and hyper triglyceridemic subjects were shown to have similar biochemical composition but different sizes and protein rich shell layer thicknesses [24] (**Figure 2D**). This suggests that LDL size and structure are not directly a consequence of composition and diet, mirroring the data found for individuals with a predisposition for sdLDL [45]. In particular, the protein rich shell layer thickness seems a very important parameter to consider since the protein conformation within this layer is determinant for further interaction with receptors and other biomolecules in the body. These structural analyses raise the following questions: are sdLDL subclasses from healthy and hyper triglyceridemic subjects equally pro-atherogenic? If not, is this due to the differences in the presence of specific apolipoproteins or post translational modifications in the protein-rich shell?

Finally, measuring total plasma cholesterol concentrations in clinics is *per se* insufficient to reliably determine the LDL atherogenic potential in a particular individual, as the LDL size-distribution component is not accounted for. The detailed understanding of LDL ultrastructure seems necessary to evaluate the atherogenicity of the individual LDL subfractions with the ultimate aim to develop effective diagnostic and therapeutic tools against CVD. Indeed, the SAXS analysis of the total LDL fraction and its subfractions shows that SAXS has the potential to discern between LDL phenotypes directly from the total LDL measurement [24] (**Figure 2D**), which could be used to extract information on the sdLDL structural parameters between subjects with a range of clinical conditions.

The role of the different HDL subclasses in the development of atherosclerosis is less studied and the results are controversial. A 2015 study found no evidence for any changes in HDL subclasses profiling for individuals with nonalcoholic fatty liver disease (NAFLD) [46], a disease associated with increased cardiometabolic risk. Another study found that HDL2b subclass was enriched in Hispanic women with LDL phenotype B [47]. Preliminary results from our group suggest that HDL main fractions and subfractions size differs between normolipidemic and

hypertriglyceridemic individuals. These contrasting results indicate that more research is needed to understand the differences in HDL subclasses across various lipid metabolism disorders. Differences in HDL and LDL subclass structure rather than their relative proportion might be relevant for their function and their role in the development of atherosclerosis. Structural studies in combination with detailed compositional analysis by means of lipidomics and proteomics, as well as post-translational modifications, are needed to advance the field.

4. Determining lipid exchange capacity by lipoproteins

As already discussed, LDL function is counteracted by HDL function, and lipid exchange is a key process for their functions. Lipid exchange is known to be independent of protein exchange [48–51]. Lipid exchange (where no net transfer takes place) and lipid transfer between lipoproteins of different types (*i.e.* between HDL and LDL) are known to occur, both *in vitro* and *in vivo*, for phospholipids, cholesterol, and sphingomyelin [52–54]. It also occurs between lipoproteins and lipid microemulsions [20, 55], lipid vesicles [56, 57], and cells [58]. The older studies used chemical analysis and focused on net changes in lipoprotein particle composition upon a given equilibration time. Therefore, these studies did not monitor the exchange processes directly. Only the work by Maric *et al.* [57] measured the kinetics of lipid exchange directly between lipoproteins and lipid vesicles making use of deuterated lipids and SANS.

It is well accepted that there are several pathways for lipid transfer and cholesterol uptake. The main pathway is through receptor-mediated endocytosis, in which lipoprotein binding to LDL receptors takes place for ApoB100 and ApoE containing lipoproteins [59, 60]. Another mechanism involves scavenger receptor class B family (SR-B) receptors [61], that mediate the selective core lipid transfer from lipoprotein particles to cells and tissues. Lipid exchange and lipid transfer can potentially occur *via* lipoprotein endocytosis and subsequent transcytosis in endothelial cells [62, 63]. Finally, direct lipid exchange or lipid transfer from lipoprotein particles to the cell membrane also occurs, as discussed above. Recently, spontaneous lipid transfer to receptor free model membranes was demonstrated by cryo-EM, fluorescence cross correlation spectroscopy and spectral imaging, regardless of lipoprotein type [64]. In particular, lipid transfer affected the model membrane properties, with HDL having the most dramatic effect in lipid packing and collective lipid diffusion.

4.1 Quantification of lipid exchange by lipoproteins

Quantification of lipid exchange and transfer is challenging, and examples were scarce in literature until recently. Browning *et al*. [65] presented a new protocol to follow interactions of human lipoproteins with model membranes using neutron reflection. HDL and LDL from healthy male adults were incubated with model phospholipid membranes in physiological-like conditions. The study showed that HDL was able to remove more lipids from the model membranes than LDL, whilst LDL deposited more lipids than HDL. The lipid exchange studies on simplified model systems mirrored the function of lipoproteins in the body!

Following reports using the same protocol showed that the inclusion of charged lipids in the model membranes led to unaffected quantity of lipids deposited but increased lipid removal by HDL [66]. The lipid exchange did not seem to affect the lipoprotein structure, as determined by SANS [57]. Then, the ability of the level of lipid unsaturation and of the presence of cholesterol to affect the lipid exchange capacity of lipoproteins was investigated [67]. The presence of acyl chain

unsaturation dramatically decreased the quantities of lipids deposited and removed by the lipoproteins, regardless of the lipoprotein type (**Figure 3**). In other words, HDL and LDL have a greater affinity for saturated rather than unsaturated lipids, though LDL to a lesser extent. The difference in ease of lipid removal between the lipoprotein types could be explained by variance in the specific protein-lipid interactions. Different conformations of ApoA1 (the main protein in HDL) were shown to have varying binding affinities to unsaturated lipid vesicles [68]. The increased ease for saturated lipid removal is likely due to the increased mobility of said lipids within the fluid model membrane: saturated phospholipids were shown to have greater mobility than their unsaturated counterparts in both gel and fluid phases [69].

The presence of cholesterol also had a large impact on the amounts of lipids exchanged and removed [67] (**Figure 3**). In the bilayers comprising saturated lipids, the presence of cholesterol decreased the amounts of lipids exchanged and removed through HDL whereas, in the case of the unsaturated lipids, the presence of cholesterol made little difference when interacting with HDL. LDL, on the other hand, did not follow the same pattern. Indeed, including 10 mol% cholesterol in the saturated bilayer increased the amount of lipids exchanged. Upon further increasing to 20 mol%, a similar value was found to that of the saturated bilayer alone. For the unsaturated model membranes, the presence of 20 mol% cholesterol also gave similar values to that of the bilayer alone. However, in terms of removal, for both the saturated and unsaturated bilayers, the presence of cholesterol increasingly reduced the quantity of lipids removed. It is possible that the presence of cholesterol may inhibit the mobility of phospholipids [70] and cause it to localise preferentially towards saturated lipids [71]. However, there were no clear systematic changes

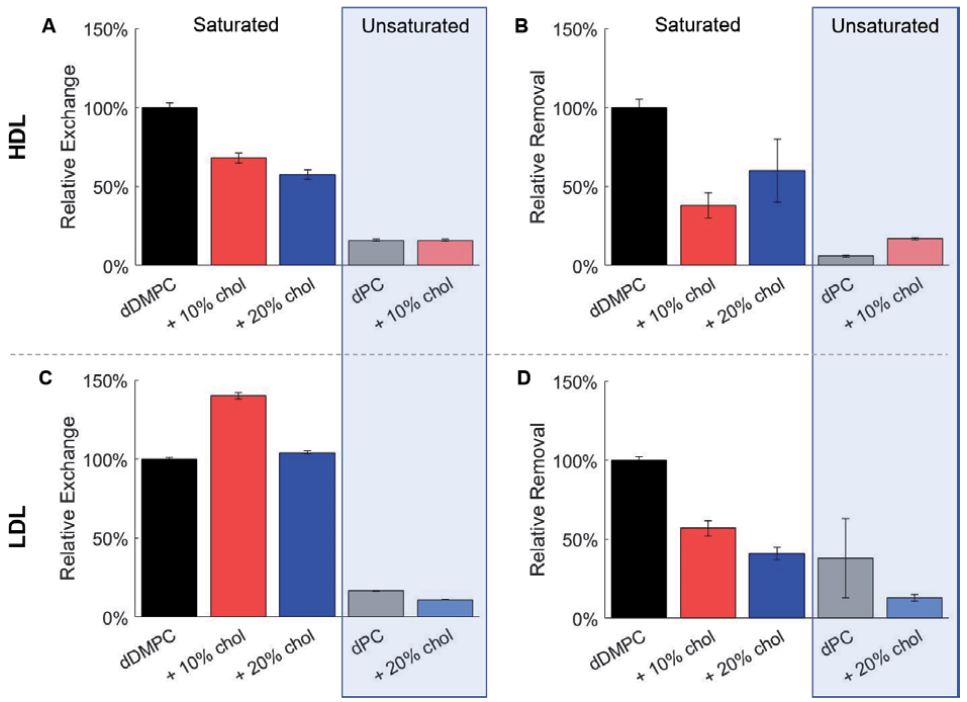

Figure 3.
Relative lipid exchange and removal for HDL or LDL particles interacting with membranes formed with saturated (dDMPC) and unsaturated (dPC) phospholipids with and without cholesterol. Results are shown for HDL (A, B) and LDL (C, D) regarding exchange (A, C) and removal (B, D). Replotted with permission from Waldie et al *2020 [67].*

when cholesterol was incorporated into the bilayer, thus it is difficult to pinpoint the exact cause of the changes seen. LDL has also been shown to preferentially bind to rafts of saturated lipid with cholesterol [72]. Since no reduction or increase in exchange was seen due to cholesterol, it is likely that the specific lipoprotein-lipid interactions determine the final quantities of lipids exchanged and removed.

To further look into this, LDL with differing compositions were used against the model membranes. It was found that with increasing density of LDL, *i.e.* increasing percentage of protein, greater levels of exchange were observed [67]. These findings can be explained by the difference in ApoB100 (the main protein in LDL) found on smaller, more dense LDL particles. Denser LDL particles contain regions with altered epitopes [73] that may house part of the lipid-binding region of LDL [74] and thus affect their binding and interactive activities.

Access to the neutron beam is limited and highly specialised personnel are needed for these experiments. Methodology based on attenuated total reflection Fourier transfer infrared spectroscopy (ATR-FTIR) seems an accessible complementary alternative method to determine lipid exchange [75], which is far more accessible and exists in many laboratories as a benchtop technique.

5. Plaque remodelling by HDL therapy

As already discussed in Section 2, and as shown by Framingham Heart Study [76, 77] and later on by the Atherosclerosis Risk in Communities study [78], there is strong inverse association between HDL-cholesterol levels and CVD risk. Changes in lifestyle, such as weight loss, increase of physical activity, reduction in saturated fat intake, and low alcohol intake can alone increase HDL-cholesterol levels by up to 20%. However, lifestyle changes alone are not sufficient to prevent and reduce mortality in atherosclerosis. Thus, over the years there have been many pharmacological interventions aimed at raising circulatory HDL-cholesterol levels while decreasing LDL-cholesterol levels [79–81]. Nevertheless, these interventions have not resulted in the expected reduction in CVD events, suggesting that HDL functionality, rather than changes in HDL levels *per se* might have a role in the disease progression. For this reason, the focus on anti-atherosclerotic solutions shifted to therapies that use the ability of HDL and its main apolipoprotein (ApoA1) to remove cholesterol from macrophages and that have thus the potential to reduce the plaques size and the steric burden at the arterial wall. One of these approaches led to the use of ApoA1 Milano, a natural variant of ApoA1 endowed with higher ability to stimulate cholesterol efflux and better anti-inflammatory and plaque-stabilising properties with respect to the wild-type protein [82]. Infusions of the Milano variant in complex with phospholipids (ETC-216) were used in patients with high CVD risk and resulted in the reduction of the atheroma volume in one study [83] but a new formulation (MDCO-216) did not result in plaque regression in patients with acute coronary syndromes and on statin treatment. Treatments based on wild-type ApoA1 have also been explored. CER-001 is a formulation of human recombinant ApoA1, sphingomyelin and phospholipid. It regressed atherosclerosis and increased RCT in mice [84] but failed to regress plaques in patients on statins with acute coronary syndrome and high plaque burden [85]. Short-term infusions of another formulation of HDL with wild-type ApoA1 and phospholipids (CSL111) showed improvement in the plaque characterisation index and quantitative coronary score, but the positive effects were accompanied by hepatotoxicity, thus the clinical development halted [86]. Later on, a new formulation (CSL-112) was developed, and its administration has been found to increase the RCT with no adverse effects in humans so far [87]. A phase III clinical study is currently ongoing and is expected

to conclude in 2022 (NCT03473223), assessing the potential benefits of CSL112 in reducing adverse cardiovascular events in subjects with acute coronary syndrome.

ApoA1 mimetic peptides have also been explored in the treatment of the adverse effects of atherosclerosis. These have the advantage to be structurally simpler than the native full-length protein, although retaining the same biological functions, and have the potential to be administered orally instead of *via* injection. One of these ApoA1 mimetic peptides is Rev-D4F, which reduced atherosclerotic lesion area and macrophage content at the lesion site in Apoe$^{-/-}$ mice, while also decreasing LDL oxidation [88]. Another ApoA1 mimetic, RG54, showed increased glucose tolerance, was able to stimulate cholesterol efflux from macrophages, and prevented the formation of atherosclerotic plaques in Apoe$^{-/-}$ mice [89]. Finally, 2F*, a photoactivatable ApoA1 mimetic peptide, was able to increase cholesterol efflux in stably transfected baby hamster kidney cells [90]. Although very promising, these peptides have been explored only from a pre-clinical point of view and their efficacy in humans and lack of side effects have still to be proved.

Another apolipoprotein that has been suggested as a good candidate for HDL therapy is ApoE. ApoE is well known for its atheroprotective properties including the ability to induce RCT from peripheral cells to the liver [91] and to stimulate cholesterol efflux from macrophages thus, in turn, preventing the formation of foam cells in the development to atherosclerosis [92, 93]. Various studies have shown ApoE to have an increased ability to protect against atherosclerosis compared to ApoA1 [94]. The Ac-hE18A-NH2 ApoE mimetic has been shown to have a superior ability than the 4F ApoA1 mimetic to reduce atherosclerotic lesions in Apoe$^{-/-}$ mice [95]. The Ac-hE18A-NH2 mimetic has also demonstrated more effective anti-inflammatory properties than the 4F mimetic [96]. Most studies that have been carried out with the use of ApoE peptide mimetics are only in the pre-clinical trial stage and have not yet been tested in clinical studies for therapeutic use [94].

The variability of the outcomes of the pre-clinical and clinical studies described above points towards the need for a deeper understanding of the functionality (RCT and fat exchange, for example) and the structure of the HDL particles before considering them as a potential therapeutic for CVD. For example, a very recent study shows that the type of lipids used in the rHDL formulation has a significant effect on their ability to mediate cholesterol efflux [97], with saturated lipids having the greatest potential for cholesterol efflux. This mimics the results for fat exchange on simplistic models presented in **Figure 3** and discussed in Section 4.1 in which saturated fats are more easily taken up by lipoproteins than unsaturated ones or those in the presence of cholesterol. Thus, not only the type of apolipoprotein but also the type of lipids used in the formulation have a fundamental role in the functionality of the rHDL and need to be further explored.

6. Conclusions

In this chapter we discussed the composition, function and structure of the two most studied lipoprotein types: LDL and HDL. In particular, we presented an updated model for determining the ultrastructure of LDL based on SAXS data that potentially enables determination of LDL phenotype from the total fraction measurement while highlighting structural differences in the small dense LDL subfraction between individuals with normal and high plasma serum triglyceride levels. Such differences, unknown until recently, may explain the different atherogenic potential of small dense LDL subfractions between different individuals and help unravel the controversies related to their atherogenic potential. Moreover, the capacity of lipoprotein fractions and subfractions to transfer lipids and cholesterol

might be linked to these structural differences. Therefore, techniques that enable quantifying lipid exchange and lipid transfer in a reproducible and systematic manner are needed. We present recent data by fluorescence spectroscopy and neutron scattering showing how model cellular membranes lacking receptors can be used in the quantification of lipid exchange and lipid transfer by lipoproteins. In particular, the methodology might be especially useful when designing HDL therapy nanoparticles which require efficient removal of both saturated lipids and cholesterol from atherosclerotic plaque. Even though HDL therapy is quite promising in pre-clinics, it still has to show its potential in clinical studies.

Acknowledgements

The authors thank the Swedish Research Council for funding.

Conflict of interest

The authors declare no conflict of interest.

Author details

Sarah Waldie, Rita Del Giudice and Marité Cárdenas*
Department of Biomedical Science and Research Center for Biointerfaces, Health and Society, Malmö University, Malmö, Sweden

*Address all correspondence to: marite.cardenas@mau.se

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Chapter 3

Lipids Abnormality and Type 2 Diabetes Mellitus: Causes and Consequences

Kan Wang and Fariba Ahmadizar

Abstract

Dyslipidemia and diabetes both are important risk factors for cardiovascular disease. Emerging evidence suggests that these two are closely related to each other, the so-called "dyslipidemia-insulin resistance-hyperinsulinemia" cycle. Recently, several new lipid subfractions, such as apolipoprotein (Apo)B, and ApoJ, have been reported to associate with insulin resistance and incident diabetes, which further claim the role of lipid in the pathophysiology of diabetes. Besides, dyslipidemia is also one of the most prevalent diabetic complications. Clinical guidelines have widely recommended lipid management among diabetic patients through lifestyle intervention and lipid-lowering medications, especially statins, to prevent cardiovascular outcomes.

Keywords: Lipid, dyslipidemia, diabetes, cardiovascular disease, statins

1. Introduction

Type 2 diabetes mellitus (T2DM) is among the most prevalent chronic disease [1], affecting approximately 463 million people in 2019, and more than 690 million are expected to be diagnosed by 2045 [2]. People diagnosed with T2DM have a 2–4-fold higher risk of developing cardiovascular disease (CVD) [3]. Despite significant advantages in the prevention strategies that lessen related risk factors, CVD remains the leading cause of morbidity and mortality in patients with T2DM [4].

T2DM and CVD both have multi-factorial etiology, and disorders of lipid metabolism is one of the coexistence features sharing by them. For the development of CVD, the cumulation of ApoB-containing lipoproteins in the arterial wall would lead to lipid deposition and an atheroma initiation, resulting in the progression of atherosclerotic plaques, and eventually atherosclerotic vascular disease [5]. Therefore, lipid-lowering drugs, such as statins, have been recommended as front-line therapy for primary prevention of atherosclerotic CVD [6], and the state-of-the-art therapy in dyslipidemia in diabetic patients [3, 7, 8]. For the potential mechanism between lipid metabolism and diabetes, one meta-analysis reported that lipid parameters, such as triglyceride (TG), and low-density lipoprotein (LDL), can reflect the risk of T2DM [9]. Other lipid subfractions, such as high-density lipoprotein cholesterol (HDL) and lipid-free ApoA-I, could also benefit glycemic control by increasing glucose uptake in skeletal muscle, improving beta-cell function, and decreasing insulin resistance through inhibiting the proinflammatory signal transduction pathways [10].

Considering the rather complex components of lipid and different directions for the associations between different lipid components and diabetes [11], more efforts are needed to elucidate the relationships between lipid profile and diabetes.

2. Lipid abnormality and incident type 2 diabetes mellitus

There are six major lipoproteins exist in blood: chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL); lipoprotein(a) (Lp(a)), and high-density lipoprotein (HDL) [12]. Dyslipidemia represents a cluster of lipid and lipoprotein abnormalities, including elevation of both fasting and postprandial TG, apolipoprotein (Apo)A, or ApoB. The prevalence of dyslipidemia is getting severer worldwide. According to the 2015 Global Burden of Disease Study, the prevalence of elevated total cholesterol (TC) (≥200 mg/dL) was highest in the WHO European Region (54% for both sexes), followed by the WHO Region of the Americas (48% for both sexes). The WHO African Region and the WHO South-East Asia Region showed the lowest percentages (23% and 30%, respectively) [13]. In China, the prevalence of dyslipidemia was 42.8% overall [14], with 4.3%, 2.4%, 14.7%, and 17.4% for the age-standardized prevalence of high TC, LDL, TG, and HDL, respectively [15].

Evidence suggests that the development of T2DM was closely associated with lipid abnormality [16–26]. A study based on the 2010–2012 China National Nutrition and Health Survey (CNNHS) reported that the prevalence of dyslipidemia was 39.9%, 46.8%, and 59.3% in participants with normal glucose, prediabetes, and T2DM [16]. Zhu et al. 's meta-analysis reported that there were positive associations between different lipid parameters and T2DM. Moreover, the standardized mean difference for the Atherogenic index of plasma (AIP, lg(TG/HDL)) is 1.78 (95% confidence interval (CI): 1.04–2.52), which is higher than for other parameters (TG: 0.93, 95% CI: 0.78–1.09; TC: 0.46, 95% CI: 0.21–0.71; HDL-C: −0.89, 95%CI: −1.18 to −0.60; and LDL-C: 0.44, 95% CI: 0.11–0.77), suggesting AIP may be more closely associated with the risk of T2DM [9]. Results from the prospective cohort study (Multi-Ethnic Study of Atherosclerosis) found that elevated total ApoC-III concentrations were associated with a higher rate of diabetes, while ApoC-III-defined HDL subspecies displayed opposing associations: HDL lacking ApoC-III was inversely associated with incident diabetes, while no association was found for HDL containing ApoC-III. Further adjustment for plasma TG as a potential intermediate attenuated these associations [17]. Besides, not only baseline cross-sectional estimated or abnormal lipid level is related, Zheng et al. found that time-dependent TG/HDL ratios were also positively associated with future incident T2DM, which supports the change patterns for these parameters during the follow-up could intricate the development of diabetes [18]. Beshars et al. evaluated the relationship between diabetes and the increment in triglyceride levels within the normal range. Their results suggest that sustained increments in rising triglyceride levels, even within the accepted normal range, might pose a cumulative risk for the development of diabetes and impaired fasting glucose [19].

Despite the aforementioned epidemiologic evidence, the precise mechanisms of the associations are complex and remain unclear. Since the hallmark of T2DM is the inability of pancreatic beta-cells to produce adequate amounts of insulin, accompanied by reduced tissue responsiveness to insulin, also known as insulin resistance. Lipotoxicity caused by dyslipidemia plays an important role in the development and progression of insulin resistance [10]. It can disturb the utilization of insulin in peripheral target tissues (such as the liver), thus affecting the amount of lipids synthesis in the liver. So, elevated blood lipid can lead to insulin resistance,

which in turn aggravates the generation of lipid metabolism disorder [27–29]. More recently, Seo et al. found that ApoJ is a novel hepatokine targeting muscle glucose metabolism and insulin sensitivity through low-density lipoprotein receptor-related protein-2 (LRP2)-dependent mechanism, coupled with the insulin receptor signaling cascade. In muscle, LRP2 is necessary for insulin receptor internalization, an initial trigger for insulin signaling, that is crucial in regulating downstream signaling and glucose uptake. Of physiologic significance, deletion of hepatic ApoJ or muscle LRP2 causes insulin resistance and glucose intolerance [30].

3. Lipid-lowering medication and incident type 2 diabetes mellitus

Lipid-lowering medication plays an essential role in the current healthcare system, not only for the optimization of the lipid profile but also to reduce cardiovascular risk [6, 12]. Recently, there is increased awareness of the possibility that lipid-lowering medications may affect glucose control and insulin resistance [31–33]. This phenomenon is reported in all classes of lipid-modifying agents, with differential effects of distinct drugs.

Some insights into this question emerged from some recent studies. Barak et al. systematically reviewed the related evidence and reported that both statins and niacin are associated with increased risk of impaired glucose control and development of new-onset diabetes, as opposed to bile-acid sequestrants which display concomitant moderate lipid and glucose-lowering effects, and fibrates (particularly the pan-PPAR agonist bezafibrate) which may produce beneficial effects on glucose metabolism and insulin sensitivity [34]. Another recently published meta-analysis, which included 163,688 nondiabetic patients from thirty-three randomized controlled trials, reported no significant association between 1-mmol/L reduction in LDL cholesterol and incident diabetes for statins or PCSK9 inhibitors. More intensive lipid-lowering therapy (defined as the more potent pharmacological strategy, such as PCSK9 inhibitors, higher intensity statins, or statins) was associated with a higher risk of incident diabetes compared with less intensive therapy (active control group or placebo/usual care of the trial). Meta-regression analysis suggested that these results were mainly driven by a higher risk of incident diabetes with statins, whereas PCSK9 inhibitors were not associated with incident diabetes ($P = 0.02$ for interaction). Thus, among intensive lipid-lowering therapies, there was no independent association between reduction in LDL cholesterol and incident diabetes [32].

The precise mechanisms for statin-induced diabetes remain unclear. However, several mechanisms have been proposed, including impaired insulin sensitivity, impaired insulin secretion, and compromised beta-cell function via enhanced intracellular cholesterol uptake due to inhibition of intracellular cholesterol synthesis by statins [34]. Recently, genetic studies have added more evidence to this. LDL-lowering alleles in *HMGCR*, which encodes the statins' molecular target, were associated with a higher risk of T2DM and higher body mass index [35]. A further larger-scale individual meta-analysis concluded that other LDL-lowering alleles, such as *NPC1L1*, *PCSK9*, *ABCG5/G8*, were also associated with a higher risk T2DM [31]. Nevertheless, it has to be stressed here that the cardiovascular benefits of statins far outweigh diabetes risk [3].

4. Type 2 diabetic dyslipidemia

Diabetic dyslipidemia is a cluster of plasma lipid and lipoprotein abnormalities that are metabolically interrelated among diabetic patients. It is mainly

characterized by increased TG levels, low HDL levels, and postprandial lipemia and contributes to the development of vascular complications [36]. Results from the 2010–2012 China National Nutrition and Health Survey (CNNHS) shown that the prevalence of dyslipidemia was 39.9%, 46.8%, and 59.3% in participants with normal glucose, prediabetes, and T2DM [16]. Another study using data from the 2010–2014 Diabetes Mellitus/Hypertension (DM/HT) study, which included 140,557 Thai adults with diabetes, reported that the dyslipidemia prevalence of 88.9% [37]. Despite the heterogeneity between different studies, the prevalence of diabetic dyslipidemia has grown gradually worldwide [4].

The pathophysiology of diabetic dyslipidemia is intricate and has not been fully understood [38]. Briefly speaking, changes in plasma lipoproteins among diabetic patients are affected by insufficient insulin function and hyperglycemia [39]. During the postprandial state, dietary fatty acids (FA) and cholesterol absorbed by the intestinal cells are incorporated as TG and cholesteryl esters into chylomicrons. In the capillary beds of adipocytes (especially in the fed state) and muscle, chylomicrons are the substrate for lipoprotein lipase (LPL), promoting lipolysis of chylomicrons TG and the release of FA. Insulin regulates LPL activity at several levels, including gene expression, protein synthesis, and secretion, and LPL is reduced in insulin-resistant individuals with T2DM with a consequent increase in plasma TG and decrease in HDL [40].

5. Type 2 diabetic dyslipidemia and cardiovascular disease

Both diabetes and dyslipidemia are important risk factors for CVD development, powered by the dyslipidemia-insulin resistance-hyperinsulinemia cycle [41]. This makes patients with diabetes dyslipidemia much more vulnerable to CVD outcomes. Results from the Multiple Risk Factor Intervention Trial (MRFIT) reported that among men who had diabetes at baseline, the absolute risk of coronary mortality at each level of blood cholesterol (for 20 mg/dL increments in TC starting from 180 mg/dL to >280 mg/dL), was 3–5 times higher in the presence of diabetes [42]. The United Kingdom Prospective Diabetes Study (UKPDS) has provided further evidence of a similarly direct and continuous association of coronary disease risk with LDL concentration. Among newly diagnosed T2DM, one mmol/L increase in LDL was associated with a 57% increased risk of myocardial infarction [43].

Many former studies have widely reported the causal association between dyslipidemia and CVD. Due to the rather complex components of lipid profile, diagnosis of diabetic dyslipidemia is not always revealed by the lipid measures used in clinical practice, as LDL levels may remain within the normal range. Therefore, it is suggested to use non-HDL levels to reflect the whole lipid spectrum [12]. The 2019 ESC/EAS Guidelines stated that ApoB analysis is recommended for risk assessment, particularly in people with high TG, diabetes, obesity, or metabolic syndrome. ApoB can be used as an alternative to LDL-C, if available, as the primary measurement for screening, diagnosis, and management [6].

Since a higher risk of atherosclerotic vascular disease in diabetic patients, lipid management has been recommended by diabetes-related clinical guidelines [3, 7, 8, 44, 45]. Consistent data have demonstrated the efficacy of statins, the first-choice lipid-lowering treatment, in preventing cardiovascular events and reducing cardiovascular mortality in patients with diabetes. A meta-analysis including 18,686 diabetic patients demonstrated that a statin-induced reduction of LDL by 1.0 mmol/L was associated with a 9% reduction in all-cause mortality and a 21% reduction in the incidence of major CV events [46]. The newly released ADA's Standards of Medical Care in Diabetes-2020 has stated that statins should be used

	Density (g/mL)	Diameter (nm)	TGs (%)	Cholesteryl esters (%)	PLs (%)	Cholesterol (%)	Apolipoproteins	
							Major	Others
Chylomicrons	<0.95	80–100	90–95	2–4	2–6	1	ApoB-48	ApoA-I, A-II, A-IV, A-V
VLDL	0.95–1.006	30–80	50–65	8–14	12–16	4–7	ApoB-100	ApoA-I, C-II, C-III, E, A-V
IDL	1.006–1.019	25–30	25–40	20–35	16–24	7–11	ApoB-100	ApoC-II, C-III, E
LDL	1.019–1.063	20–25	4–6	34–35	22–26	6–15	ApoB-100	
HDL	1.063–1.210	8–13	7	10–20	55	5	ApoA-I	ApoC-II, C-III, E, M
Lp(a)	1.006–1.125	25–30	4–8	35–46	17–24	6–9	Apo(a)	ApoB-100

Apo: apolipoprotein; HDL: high-density lipoprotein; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; Lp(a): lipoprotein(a); PLs: phospholipids; TGs: triglycerides; VLDL: very-low-density lipoprotein.

**Adapted from: "2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk." By Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. Eur Heart J. 2020;41 [1]:111–88.*

Table 1.
Physical and chemical characteristics of human plasma lipoproteins.*

Target	Statin treatment
ADA	
For patients with diabetes aged 40–75 years without atherosclerotic cardiovascular disease, use moderate-intensity statin therapy in addition to lifestyle therapy.	
For patients with diabetes aged 20–39 years with additional atherosclerotic cardiovascular disease risk factors, it may be reasonable to initiate statin therapy in addition to lifestyle therapy.	
In patients with diabetes at higher risk, especially those with multiple atherosclerotic cardiovascular disease risk factors or aged 50–70 years, it is reasonable to use high-intensity statin therapy.	
In adults with diabetes and a 10-year atherosclerotic cardiovascular disease risk of 20% or higher, it may be reasonable to add ezetimibe to maximally tolerated statin therapy to reduce LDL cholesterol levels by 50% or more.	
ESC/EASD	
In patients with T2D at moderate CV risk, an LDL-C target of <2.6 mmol/L (<100 mg/dL) is recommended.	Statins are recommended as the first-choice lipid-lowering treatment in patients with DM and high LDL-C levels: administration of statins is defined based on the CV risk profile of the patient and the recommended LDL-C (or non-HDL-C) target levels.
In patients with T2D at high CV risk, an LDL-C target of <1.8 mmol/L (<70 mg/dL) and LDL-C reduction of at least 50% is recommended.	
In patients with T2D at very high CV risk, an LDL-C target of <1.4 mmol/L (<55 mg/dL) and LDL-C reduction of at least 50% is recommended.	
CDS	
The primary goal is to reduce LDL-C to the target (very high risk of ASCVD: <1.8 mmol/L, high risk of ASCVD: <2.6 mmol/L).	Statins are the preferred lipid-lowering drugs. Lipid-lowering therapy should start with a moderate-intensity statin, and the dose should be adjusted according to individual response to medication and tolerability.
LDL-C reduction by ≥50% may be used as an alternative target in the event of high baseline LDL-C and failure to reduce LDL-C to the target after 3 months of standard lipid-lowering therapy.	
JDS	
The primary goal of antidyslipidemic therapy is to control the LDL-C level to <120 mg/dL in patients without a history of coronary artery disease.	Statins are the agents of choice for hyper-LDL-C in patients with diabetes.
The control goal for fasting triglyceride (TG) is <150 mg/dL.	
The control goal for HDL-C is ≥40 mg/dL.	

Table 2.
Guidelines for the management of diabetic dyslipidemia using statin.

for both primary and secondary CVD prevention among diabetes. The detailed guideline are listed according to age groups: for 20–39 years-old diabetic patients with atherosclerotic cardiovascular disease risk factors, statin therapy is highly recommended in addition to lifestyle therapy; for patients with diabetes aged 40–75 years without atherosclerotic cardiovascular disease, use moderate-intensity statin therapy (lowers LDL by 30–49%) in addition to lifestyle therapy; while for patients aged 50–70 years with diabetes, high-intensity statin therapy (lowers LDL by ≥50%) is recommended [8]. The 2013 ACC/AHA guideline emphasized statin therapy recommended for all patients with diabetes 40 to 75 years of age-independent of baseline cholesterol [47].

Despite the CVD protective effect among diabetic patients, statin therapy has been associated with new-onset T2DM [31, 32]. A former study reported that for every 40 mmol/L reduction of LDL by statins, conversion to T2DM is increased by 10% [48, 49]. Nevertheless, the benefits in terms of cardiovascular event reduction

greatly exceed the risks of statin therapy, and this has been confirmed in patients at low cardiovascular risk [46] (**Tables 1** and **2**).

6. Conclusion

Complex lipoprotein metabolism abnormalities could present both in the development and progression of type 2 diabetes, which indicates that lipid management can prevent cardiovascular complications among diabetic patients and involve in the prevention of diabetes. Epidemiological studies suggest that lipid components could be a marker for diabetes prediction, though it is still uncertain which lipid markers are of the most clinical value. Lipid control using a lipid-lowering medication, such as statins, could reduce CVD risk among the general population also diabetic people. However, it is necessary to consider statin diabetogenicity in clinical practice when the statin is indicated.

Conflict of interest

The authors declare no conflict of interest.

Author details

Kan Wang and Fariba Ahmadizar*
Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands

*Address all correspondence to: f.ahmadizar@erasmusmc.nl

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Chapter 4

Dyslipidemia and Endocrine Disorder

Mezgebu Legesse Habte, Etsegenet Assefa Beyene and Teka Obsa Feyisa

Abstract

Dyslipidemia is one of the most common risk factors for the most prevalent and fatal non-communicable diseases (NCDs); cardiovascular disease (CVD), cancer, chronic respiratory disease and diabetes and other. According to world health organization (WHO) report effective management of dyslipidemia can reduce incidence and mortality rate by NCDs up to 30%. Dyslipidemia negatively affects every vital organ; liver, kidney, heart, brain, lung and others in number of ways. In short dyslipidemia is defined as disorder of lipoprotein metabolism and it could be either hypolipoproteinemia or hyperlipoproteinemia. Endocrine disorder, poor feeding habit, physical inactivity and other factors are responsible for existence of dyslipidemia. Lipocrinology which studies about interrelationship between lipid metabolism and endocrine function in normal and abnormal condition is getting essential. Currently number of studies explain that dyslipidemia induce endocrine dysfunction and the reverse is also possible. In addition, biochemical assessment of dyslipidemia is used to monitor clinical course and progress of endocrinological diseases. Similarly, biochemical analysis of hormones helps to assess the outcome of antidyslipidemic drugs and prognoses the condition dyslipidemia. Most commonly dyslipidemia coexist with type 2 diabetes, obesity and metabolic syndrome. Abnormal distribution and accumulation fat in the body leads to deranged different hormones and factors secretion like adipokine, thereby contributing to chronic inflammation and lipotoxicity. Therefore, detailed and up-to-date review about lipid metabolism disorder and endocrine function are so essential in medicine and health science to have good service to community.

Keywords: dyslipidemia, endocrine, hormone, lipocrinology

1. Introduction

Different scientific journal define dyslipidemia in different ways but the idea is the same. Dyslipidemia defined as unhealthy levels of one or more kinds of the following lipid particles in the blood; high-density lipoprotein (HDL), low-density lipoprotein (LDL), intermediate density lipoprotein (IDL), very low-density lipoprotein (VLDL), triglycerides, cholesterol and others. In other words, dyslipidemia could be explained as disorder of lipoprotein metabolism and the condition could be either hyperlipoproteinemia or hypolipoproteinemia [1]. Higher plasma level of atherogenic and immunogenic lipid particles such as LDL, triacylglycerol, cholesterol and small density lipoprotein and lower plasma level of HDL are indicators of dyslipidemia [2].

Lipids are water-insoluble heterogeneous organic molecules and can be extracted from tissues by nonpolar solvents. As a result of its nature body lipids are generally found compartmentalized, and could exist as membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein. Its chemical nature makes lipids as a major source of energy for the body and hydrophobic barrier. In addition, lipids act as a precursor for synthesis important biomolecules like fat-soluble vitamins, prostaglandins and steroid hormones and others [3].

An endocrine gland secretes hormone into the blood and traveled to target cells or tissues located elsewhere in the body where they have specific receptor. Accordingly target cell and tissue produce unique response. Hormones are responsible to regulate most biological activity in the body essential for survival and reproduction [4]. Every physiological activity; sleeping, drinking, feeding, growth, aging and others needs the involvement of hormones [5].

Hormones like leptin, insulin and adiponectin influence lipid metabolism and plasma lipid profile level which is associated with obesity. Leptin and adiponectin regulate lipid metabolism and increase fatty acid oxidation in the peripheral tissue by activating adenosine monophosphate (AMP) dependent kinase pathway. In addition, adiponectin increase insulin sensitivity to tissue thereby it decreases triacylglycerol and downregulate its plasma level [6]. Currently number of studies explain that dyslipidemia induce endocrine dysfunction and the reverse is also possible. In addition, biochemical assessment of dyslipidemia is used to monitor clinical course and progress of endocrinological diseases. Similarly, biochemical analysis of hormones helps to assess the outcome of antidyslipidemic drugs and prognoses the condition dyslipidemia [7].

1.1 Dyslipidemia among non-communicable disease

Lipids are one of the most important macromolecules; used by our body as energy source, structural components of cell membrane, precursor for synthesis of steroid hormones and fat-soluble vitamins etc. There are also essential fatty acids like linolic and linolenic which are necessary for normal biological activity and human body cannot synthesis them. Knowing lipid biochemistry and its metabolic disorder (dyslipidemia) is necessary in understanding the biomedical areas of non-communicable disease like hypertension, diabetes, cancer, cardiovascular disease, obesity, nutritional value of unsaturated fatty acids and others [8].

According to the world health organization 2015 report non-communicable disease (NCDs) are responsible for up to 70% of deaths and these are hypertension, cardiovascular diseases, cancer, diabetes and chronic respiratory diseases. Majority of NCDs cases and deaths are observed in low- and middle-income countries. The report shows that tobacco, insufficient physical activity, harmful use of alcohol, unhealthy diet, raised blood pressure, overweight and obesity, raised cholesterol, cancer-associated infections and others are the major risk factors for NCDs [9, 10].

Number of studies showed that dyslipidemia used as a biomarker and risk factor for different NCDs and it is highly associated with one or more hormone disfunction. For example, deficiency or defect in insulin action among diabetes induce dyslipidemia and it is one of the major risk factors for cardiovascular disease. Dyslipidemia is the common finding among different types of diabetes. Across suctional study done by Hrishow et al. found that 73% male and 71% female of diabetic patients were dyslipidemic [11]. Another similar study done by Bekele S. et al. in Ethiopia revealed that 65.6% of diabetic patients were dyslipidemic. Plasma level of LDL, total cholesterol and triacyclglycerols were higher while HDL was lower among diabetic patients [12]. Similarly, number of recent studies showed that dyslipidemia has significant association with cancer and progress of the disease.

Lipids are major constituents of cell and disorder in lipid metabolism can affect the normal integration of cell which may leads to cancerous. On the other side treatment for cancer patients specially chemotherapy induce dyslipidemia. In addition, oxidative modification of lipoprotein induces different inflammatory pathways and this enhance cell proliferation and migration and inhibit normal cell apoptosis [13]. Review done by Cedó L. et al. indicated that higher total serum cholesterol level is linked with higher risk of colorectum, colon, prostate and testicular cancer and lower risk of stomach, liver and hematopoietic and lymphoid tissues cancer. There was positive association between serum triglycerides and esophageal, colorectal, lung, renal, thyroid cancer [14]. Effective management of cholesterol may help to control the risk of cancer cases. Study by Macleod LC et al. stated that elevated LDL and impaired fasting glucose are highly associated with prostate cancer and management on them help to get better treatment outcome at the end [15].

Number of studies explained that dyslipidemia is a common risk factor for different types of cardiovascular disease. A 6 year follow up prospective cohort study done by Hedayatnia M et al. explained that serum LDL-C, TC, and TG levels were positively associated with the risk of total CVD events and the primary target of lipid-lowering therapy with statin is to reduce risk of CVD [16, 17]. Events of dyslipidemia occurred among patients with different infectious disease like HIV and TB increase risk of atherosclerosis and finally leads CVD. The infectious disease itself and the drugs given for that are responsible to induce dyslipidemia [18, 19].

1.2 Effect of dyslipidemia on vital organs

Survival of living organism like humans relies on wellbeing of vital internal organs; brain, liver, heart, lung and others. An organ which exists in most multicellular organisms is a collection of tissues joined in a structural unit to serve a common function. Normal and functional liver is responsible for healthy metabolism of lipids. Study done by Unger LW *et al* showed that liver health is a major determinant of dyslipidemia patterns and prevalence. All etiology that induces advanced chronic liver disease significantly decrease total cholesterol levels when compared to normal [20]. Dyslipidemia can cause fatty liver disease and the reverse also possible. A multicenter retrospective cross-sectional study done for 5 years by Méndez-Sánchez N *et al* showed that dyslipidemia was the very common risk factor which is directly associated with advanced liver disease and cirrhosis [21].

Brain is one of very important vital organ for normal function of all other body organ and organ system. Studies indicated that dyslipidemia can affect the integrity and function of brain and significantly associated with number brain disorder like Alzheimer's Disease. A one year follow up study done on 36 subjects with Alzheimer's Disease by Bowman GL. *et al* confirmed that dyslipidemia was highly prevalent and more than 75% of them showed blood brain barrier (BBB) impairment. According to this study triglyceride and HDL cholesterol which have a role in maintaining BBB integrity were significantly higher among subjects with BBB impairment [22]. A Meta-Meta-Analysis done by Vazquez OS *et al* conclude that LDL-C cholesterol levels significantly increase the risk for development of Alzheimer's disease. Hypercholesterolemia affect the normal function of vascular system and have neurotoxic effect thereby induce dementia and cognitive loss in Alzheimer's Disease patients. In addition, coronary heart disease and carotid artery atherosclerosis raised as a result of hypercholesterolemia and that can lead to cognitive disorder by causing cerebral embolism or hypoperfusion which are associated with Alzheimer's Disease incidence and prevalence [23]. Studies also showed that there is significant association between high total cholesterol and LDL-cholesterol with different types of stroke as a result people with hypercholesterolemia have advised to take potent lipid

lowering drugs mainly statin to reduce risk of stroke. But there is an evidence that low level of total cholesterol can induce hemorrhagic stroke. Cohort study for one year on Patients with Transient Ischemic Attack done by Sirimarco G. *et al* showed that atherogenic dyslipidemia was significantly associated with intracranial artery stenosis and higher risk of early recurrent stroke. In general disorder in lipid metabolism have a complex relationship with cerebrovascular disease [24, 25].

Bones which are found in different shapes and sizes have many roles in wellbeing life and its health has intimate association with normal metabolism of lipids. Evidence indicated that highly increased adipocyte can be independent risk factor for osteoporosis and disorder of bone metabolism [26, 27]. Oxidation products of lipids as a result of hyperlipidemia can be accumulated in the subendothelial spaces of vasculature and bone and these affect the normal developments of bone negatively. Study done on mice by Pirih F *et al* explain as dyslipidemia is one risk factor for thyroid dysfunction and impairs regeneration and mechanical strength of bone [28]. Another study done on rabbit to study the effect of hyperlipidemia on quality and quantity of bone and wound healing condition showed that hyperlipidemia negatively affect bone implant stability and pre-implant stability [29].

1.3 Dyslipidemia induce endocrine dysfunction

Disorder in lipid metabolism is a multifactorial problem; genetic and environmental risk factors are very important modulators. Number of studies explain that dyslipidemia has significant association with age, sex and educational status and others. Dyslipidemia affect the normal health in number of ways and the mechanism is complex. Diabetes, cardiovascular disease, hypertension and other noncommunicable disease have direct or indirect relation with dyslipidemia. And most of non-communicable disease have endocrine bases; as a result, deep analysis of endocrine disorder and dyslipidemia is very crucial. For example, in cancers of the breast, prostate, testes, cervices, pancreas, thyroid and others patients have abnormal lipid profile and all the cases have hormonal problems. A cross sectional study done by Altahir WHM *et al* showed that triacylglycerol was significantly higher while cholesterol was lower among hyperthyroidism patients [30]. Also, other cross-sectional study done by Khan MAH *et al* indicate as there is strong association between dyslipidemia and hypothyroidism [31].

There is clear evidence as chronic elevation of triacylglycerol in the blood cause islet alpha- and beta-cells dysfunction. In the general obese population; fasting level of insulin and glucagon have positive correlation with lipid parameters. Level of glucagon is clear informative of glucose tolerance and the mechanism how elevated fatty acids affect islet-cells is through inactivation of G-protein coupled receptor called Free fatty acid receptor 1 (FFAR1) [32]. Cholesterol can modulate different metabolic pathways like signal transduction and gene expression in numbers of ways. Many studies showed that cholesterol level in the blood affect the secretion and function of insulin from beta-cell of pancreas. Animal model study done by Hao M *et al* found that elevated level of cholesterol can significantly reduce insulin secretion and the condition can be restored with management of blood cholesterol. The suggested mechanism for this is through manipulation of neuronal nitric oxide synthase dimerization [33].

Steroid hormones like sex hormones are synthesized from cholesterol, so the normal concentration of cholesterol is responsible for normal sexual development of an individual. Production, secretion and degradation of factors that are involved in function of sex hormones like Sex hormone-binding globulin (SHBG) are regulated with lipid panels of individual. Study done by Park G *et al* indicated that dyslipidemia negatively affect the level SHBG and these can disturb the normal developments children at pubertal stage [34].

1.4 Endocrine disorder risk for dyslipidemia

An endocrine system secretes specific chemicals, called hormones into the blood and travel through the blood to target cells or tissues that they induce a particular response or action. Hormones involved in regulation of biological activities like growth, reproduction, metabolism of biomolecules (protein, lipids, carbohydrate, nucleic acids and others). Endocrine disorders are among the most common medical problems faced by individual in both sexes. Defect in synthesis, storage, secretion and action of hormones can seriously affects normal biological activities of individuals in number of ways. One of the most important system affected as a result of endocrine disorder is lipid metabolism and these in turn risk for developments of different non-communicable diseases. Diabetes mellitus is one common endocrinological disorder and it leads to dyslipidemia. Cross-sectional study done by Xi Y *et al* showed that all types of diabetes and postmenopausal hormonal changes are positively correlated with dyslipidemia [35]. Hormones regulate expression of lipoprotein receptors, apoproteins, enzymes and control circulating triacylglycerol and glucose. Typical hormones involved in modulating lipid metabolism are leptin, insulin, adiponectin, glucagon, catecholamines, cortisol and growth hormone and any disorder on these hormones' synthesis, storage, secretion and action leads to dyslipidemia. Body lipid synthesis and degradation are under control of the above hormonal activity [36, 37]. Number of studies confirmed that dyslipidemia is the most common disorder among diabetes especially type 2 diabetes. As a result, people with diabetes should monitor their blood lipid levels regularly and get nutrition education periodically to prevent and control dyslipidemia [38].

Higher level of growth hormone and factors like insulin like growth factor-1(IGF-1) leads to a medical problem called acromegaly. And these conditions alter the metabolism of biomolecules and activity of other hormones like insulin thereby risk for cardiovascular disease mainly by inducing dyslipidemia. Study done by Keskin H *et al* explained that LDL-C, triglyceride, IGF-1 level was significantly higher among acromegaly patients than control groups while HDL-C level was lower. In addition, growth hormone has positive correlation with LDL-C and triglyceride and negative correlation with HDL-C level [39].

One important steroid hormone called cortisol is responsible to regulates a wide range of vital biological processes including metabolism of biomolecules and immune response throughout the body. Cortisol is made in the cortex of the adrenal glands and released into the blood. Most of body cells have receptor for cortisol so that it produces different effect. Cortisol secretion regulated by close interaction of hypothalamus, pituitary gland and adrenal gland. One of the metabolic process regulated by cortisol is lipid metabolism. Study done by Djurhuus C. B. showed that cortisol is a potent stimulus of lipolysis in both femoral and abdominal adipose tissue [40]. Hypersecretion of cortisol which resulted from altered function of hypothalamus, pituitary gland and adrenal gland can leads to dyslipidemia. The mechanism by which cortisol disturb lipid metabolism is vary; initiate lipolysis, free fatty acid production and turnover, VLDL synthesis and fatty accumulation in liver and adipose tissue. Modulation of insulin sensitivity and adenosine monophosphate kinas (AMPK) activities are the key by which glucocorticoid-induced dyslipidemia will develop. In addition, defects of the glucocorticoid receptor affecting cortisol sensitivity is involved in lipid metabolism disorder and risk for cardiovascular disease [41].

Similarly, sex hormones are responsible in regulating metabolism lipids and any disorder regarding these sex hormones leads to dyslipidemia. In study done by Yeasmin N *et al* showed that serum total cholesterol and triacylglycerol level were significant higher in postmenopausal women than premenopausal women and they have negative correlation with serum estrogen as a result of this

postmenopausal women are more prone to have CVD and associated complications and recommended to monitor their lipid profile regularly [42, 43].

1.5 Assessment of lipid profile as marker for endocrine disease

Lipids are a group of fats and fat-like substances that are important constituents of cells and sources of energy. Assessments lipid panel is simply qualitative and quantitative determination of the specific lipid parameters in the blood. Lipid profile test commonly performed to evaluate cardiovascular diseases including coronary heart disease (CHD), myocardial infarction (MI), coronary insufficiency, angina, ischemic stroke, hemorrhagic stroke, transient ischemic attack, peripheral artery disease (PAD) and heart failure. Directly or indirectly all the above cases related with endocrine disorder. In addition, evaluation of TG/HDL-C and TC/HDL-C ratios are useful markers of metabolic syndrome with high predictive value in general population. Studies showed that LDL-C, total cholesterol, triglyceride and apolipoproteinB (apoB) can serves as biomarker Cushing syndrome [44]. Similarly, one or more lipid profile abnormality is common prior to type 2 diabetes [45]. In general, in most of endocrine disease dyslipidemia is common and physician who suspect his patients have hormonal problem should order lipid profile test [46].

1.6 Conclusion

Dyslipidemia is an abnormal amount of lipids commonly triglycerides, cholesterol LDL-C, HDL.C and other in the blood and it is highly prevalent among patients with noncommunicable disease. Each and every lipid profile parameter abnormality has direct or indirect relation with endocrine disorder. Even though it needs more extended research disorder in lipid metabolism induce hormonal dysfunction and the reverse also possible.

Authors' contributions

Mezgeu Legesse Habte drafted the paper and write the literature review. Etsegenet Assefa and Teka Obsa assisted in guidance, critical assessment and peer review of the writing. All authors have given their final approval of this version to be published. All authors read and approved the final manuscript.

Funding

This review chapter is not funded by any person or organization (not funded).

Conflict of interests

The authors declare that they have no competing interests.

Ethical statement

Availability of data and material: All necessary data and materials related to the chapter are included in the chapter.

DOI: http://dx.doi.org/10.5772/intechopen.95756

Author details

Mezgebu Legesse Habte[1*], Etsegenet Assefa Beyene[2] and Teka Obsa Feyisa[1]

1 Department of Biochemistry, Harmaya University, School of Medicine and College of Health Sciences, Ethiopia

2 Department of Biochemistry, College of Health and Medical Sciences, Addis Ababa University, Addis Ababa, Ethiopia

*Address all correspondence to: mezgebulegesse@gmail.com

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Chapter 5

Cholesterol Recognition Motifs (CRAC) in the S Protein of Coronavirus: A Possible Target for Antiviral Therapy?

Antonina Dunina-Barkovskaya

Abstract

Some interactions of enveloped viruses with the host cell membrane have a cholesterol-dependent component, which may account for clinical manifestations of the infectious disease and can be used for the development of antiviral drugs. These cholesterol-dependent interactions can be mediated by cholesterol-recognition amino-acid consensus (CRAC) motifs present in viral proteins. The S protein of the SARS-CoV and SARS-CoV2 coronaviruses contains CRAC motifs that can be involved in the process of virus entry into the cell. Besides, during viral envelope formation, CRAC motifs can be responsible for binding of cell membrane cholesterol, leading to depletion of cell membrane cholesterol and subsequent malfunctioning of cellular cholesterol-dependent proteins, destabilization and permeabilization of cell membranes and, ultimately, to the death of infected cells. Understanding the mechanisms of cholesterol-dependent virus–cell interactions and the role of CRAC-containing viral proteins in the pathogenesis of the disease can serve as the basis for the development of new drugs that prevent both coronavirus entry into the cell and the damage of the infected cell during the viral morphogenesis. The target for such drugs can be the S-protein/cholesterol interface. CRAC-containing peptides derived from viral proteins may be among these agents. These peptides can also be used as experimental tools to study cholesterol-dependent virus–cell interactions.

Keywords: peptides, cholesterol-recognition motif (CRAC), CRAC-containing peptides, coronavirus, S-protein, SARS-CoV2, COVID-19

1. Introduction

The COVID-19 pandemic caused by the SARS-CoV2 coronavirus has resulted in almost hundred of millions of infections and about two millions of deaths in 2020 [1, 2]. According to the WHO data as of January 4, 2021, there have been 85 929 428 confirmed cases of COVID-19 reported to WHO, including 1 876 100 deaths [2]. It has been shown that in patients with laboratory-confirmed COVID-19, clinical results correlate with the presence of concomitant diseases, among which hypertension and diabetes mellitus, as well as old age, atherosclerosis, cardiovascular and cerebrovascular diseases worsening prognosis [3–9]. Notably, these factors are commonly associated with the impairments in the lipid/cholesterol metabolism and transport or are their direct consequence [3–5].

At present, a lot is known about the coronavirus SARS-CoV2. It is an enveloped single-stranded RNA virus belonging to the Betacoronavirus genus of the Coronaviridae family. The virus contains a 30 kb genome encoding four major viral structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins [9, 10]. According to modern concepts, the cellular receptor for S protein is angiotensin converting enzyme 2 (ACE2); binding of the S protein to this receptor allows the internalization of the virus and triggers the disease. Significant progress has been made in the development of vaccines against SARS-CoV2 and mass vaccination of people in different countries begins [11, 12]; this gives hope that the pandemic will stop. However, the issues of treating patients with various forms of COVID-19 and the development of drugs based on knowledge of the mechanisms underlying this pathology still need to be addressed. This chapter is mainly focused on the lipid aspects of the interactions of coronaviruses with host cells and, in particular, draws attention to the fact that interactions with host cells of many enveloped viruses, including coronaviruses SARS-CoV and SARS-CoV2, are cholesterol dependent. Moreover, these interactions lead to significant and potentially deleterious alterations in the cholesterol status of the infected cells. These cholesterol-dependent processes play a significant role both at the stage of the virus entry and during the development of severe respiratory syndrome (SARS) and other health problems caused by coronaviruses SARS-CoV and SARS-CoV2. Therefore, understanding this component is necessary for the development of additional approaches both to prevention and treatment of these diseases, and the attempts in this direction are being made (e.g., [13–15]). This review focuses on the fact that the coronavirus S protein, which is involved in cholesterol-dependent virus–cell interactions during entry and replication stages, contains the so-called cholesterol recognition amino-acid consensus (CRAC) motifs [16, 17] that can actually mediate these interactions. A hypothesis is put forward suggesting that binding of cell membrane cholesterol by CRAC-containing S protein (and possibly by other viral proteins) and subsequent removal of cholesterol from intracellular membranes by newly formed viral particles can affect normal functioning of cellular cholesterol-dependent proteins (receptors, ion channels, enzymes, etc.) and can eventually cause cell death due to destabilization and permeabilization of cell membranes. This deteriorating effect of CRAC-containing viral proteins can be counteracted by agents that prevent binding of membrane cholesterol to viral proteins and/or compensate for the membrane cholesterol depletion produced by the forming viral particles. It is possible that specially designed CRAC-containing peptides that specifically block interactions of S protein with cholesterol can expand the range of antiviral agents.

2. Some interactions of enveloped viruses with host cells are cholesterol-dependent

The viral life cycle includes four steps: entry, replication, assembly, and egress [18–21] (**Figure 1**). At the entry step, an enveloped virus binds to a target receptor, the viral envelope fuses with the host cell membrane, and the viral nucleic acids are released into the cytoplasm. At the replication step, the nucleic acid is replicated in cytoplasmic replication organelles and viral proteins are synthesized. At the assembly step, viral proteins and nucleic acids are packed into a viral particle and the viral envelope is formed. At the egress step, mature viral particles leave the cell through the cellular membrane [18–21].

Some interactions of enveloped viruses with the cell in the course of penetration and during assembly, budding, and exit of the virus from the cell are known to

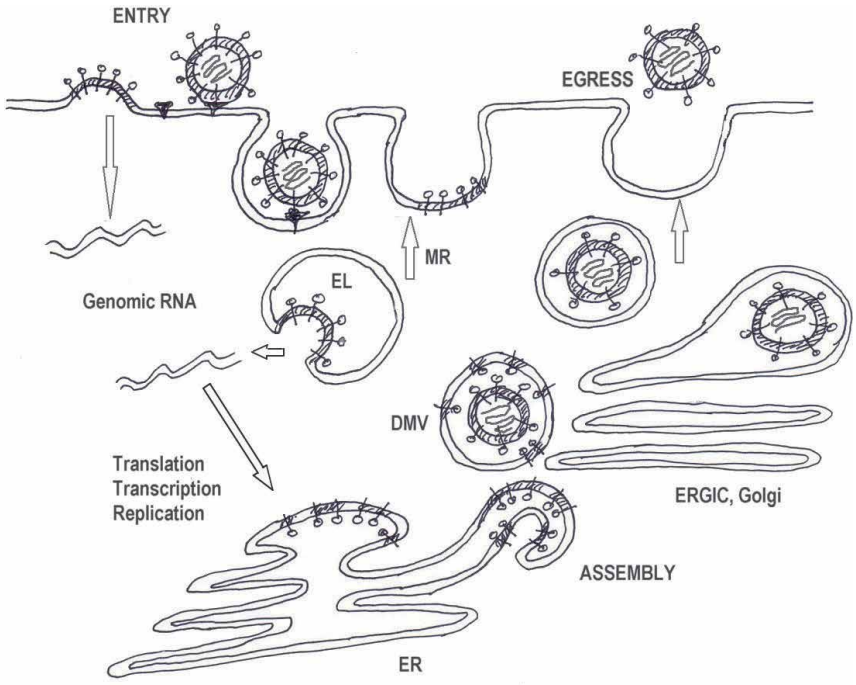

Figure 1.
Life cycle of an enveloped virus as exemplified by coronavirus (based on [18–21]). To enter a cell, virus binds via S protein to the receptors on the host cell and the viral envelope fuses with plasma membrane or membrane of endolysosome (EL) and releases the genomic nucleic acid into the cell cytoplasm. Viral membrane is shaded. Note that viral envelope remains in cell plasma membrane both after direct fusion and after endosomal membrane recycling (MR). At the replication stage, replication–transcription complex is formed and viral proteins and copies of RNA are produced. At the assembly stage, viral particles are formed from nucleocapsid (genomic RNA and N protein) and intracellular cell membranes (endopasmic reticulum (ER) and/or ER-to-Golgi intermediate compartment (ERGIC)), containing viral membrane-associated proteins (S, M, and E). During the assembly process, double-membrane vesicles (DMV) are formed. At the exit stage, viral particles are released by secretory pathway.

depend on the presence of cholesterol and lipid rafts in the membranes of the host cells [21–26]. This has been shown for immunodeficiency viruses (HIV) [27–31], influenza [32–37], herpes [38], Newcastle disease virus [39], and rotavirus [40], as well as hepatitis C virus (HCV) [41–43] and some other viruses of the Flaviviridae family (Yellow fever virus, Zika virus, Dengue virus, West Nile virus [44, 45]). For example, the vital need of cholesterol for replication of hepatitis C virus (HCV) was shown by different methods in [41, 43]. Lipid withdrawal from the medium considerably suppressed the virus replication, which was restored to normal levels upon addition of exogenous LDL. Moreover, virus replication was suppressed by knockdown or pharmacological inhibition of Niemann–Pick type C1 protein (NPC1) – cell protein mediating the endosomal cholesterol transport [41, 43].

The cholesterol dependence of virus–cell interactions has also been demonstrated for various coronaviruses [14, 15, 46–52], including SARS-CoV and SARS-CoV2 [50–52]. Li et al. 2007 [50] reported that the production of SARS-CoV particles released from the infected Vero E6 is notably suppressed following cholesterol depletion by cell pretreatment with methyl-β-cyclodextrin (mβCD), and the addition of cholesterol to the culture medium reversed this effect. Later, Glende et al. 2008 [51] showed that the removal of cholesterol from cell membranes using mβCD reduces the efficiency of infection of cells not only with the SARS-CoV but also with a non-replicating pseudotype of vesicular stomatitis virus containing the surface glycoprotein S of the SARS-CoV virus (VSV-ΔG-S), which confirms the key

role of the S protein in the virus entry. The authors also reported that the cellular receptor of the SARS-CoV virus, angiotensin-converting enzyme (ACE2), is co-localized with Flotilin2 and LAMP2, the protein markers of the detergent-resistant membrane domains (rafts) [51].

The issues concerning the importance of the host cell membrane lipids, rafts, and cholesterol at different stages of the virus life cycle have been addressed in numerous comprehensive reviews, and the dependence of the viral life cycle on cellular cholesterol, as well as the impact produced by viruses on cellular lipids and cholesterol in particular is regarded as a basis for antiviral therapy [15, 30, 31, 45, 53]. For example, cholesterol-lowering treatments are considered as a possible prophylactic or preventive measures [45, 54]. However, alterations in the cell lipid status produced by viruses that have entered a cell impose more complex requirements on potential medicines.

3. Virus-induced modulations of the lipid composition of cell membranes. Formation of viral envelope can result in a hazardous decrease in the cholesterol content in cell membranes

Viruses not only depend on cholesterol but also significantly modulate the lipid composition of cell membranes [53, 55–63]. This occurs both at the stage of virus internalization and during the synthesis of viral proteins and intracellular assembly of new viral particles. The consequences of these changes can determine the clinical course and severity of the disease. The release of the gene material of many enveloped viruses into the cytoplasm of the cell occurs by fusion of the viral envelope with plasma membrane or with membranes of late endosomes (endolysosomes) formed during receptor-mediated endocytosis [18–21, 64–70]. The inclusion of viral envelopes into the host cell membrane (either after direct fusion of after endosomal membrane recycling) should change both the lipid and protein composition of the cell membrane and cause rearrangements in the lipidic milieu and antigenic profile of the host cell membrane (**Figure 1**). Further, at the stage of the assembly of new viral particles, their envelopes are formed from cellular membranes [14, 15, 45, 55, 57–65, 67, 68]. Some viruses bud from the plasma membrane (e.g., togaviruses, rhabdoviruses, paramyxoviruses, orthmyxoviruses, and retroviruses, including HIV), others use the endoplasmic reticulum (ER) (coronaviruses and flaviviruses) or/and a Golgi complex (bunyaviruses), some (e.g., herpes virus) have more complicated budding scenario [59–62]. The formation of the viral envelopes can involve lipid sorting and, in particular, accumulation in the viral envelope of cholesterol and sphingolipids that are acquired from the host cell membranes [33, 61–65, 69, 70]. For example, HIV-1 selectively buds from membrane domains enriched in cholesterol and sphingolipids (rafts); as a result, host cell rafts become a viral coat and the level of cholesterol and sphingolipids and the cholesterol/phospholipids ratio in the viral envelope is higher than in the plasma membrane where they originate, and also notably higher than in the intracellular membranes [53, 61, 62, 65]. Another example – bovine viral diarrhea virus (BVDV) of the Flaviviridae family budding from the endoplasmic reticulum (ER): the content of cholesterol, sphingomyelin, and hexosyl-ceramide in the BVDV particles was shown to be more than twofold higher than in the infected cells [69]. As the cholesterol concentration in the ER is significantly (several times) lower than in the plasma membrane [71–74], the loss of cholesterol due to the formation of viral envelopes can be destructive for the ER membranes.

For lipid sorting necessary for the virus envelope formation, various mechanisms are used to manipulate synthesis, metabolism, and transport of host lipids,

cholesterol in particular, and lead to significant changes in the lipid status of the host cell. HIV-1 infection is known to induce various alteration of cellular lipids, including increased cholesterol synthesis and uptake [75], suppressed cholesterol efflux [76], as well as a shift in phospholipid synthesis to neutral lipids and peroxidation of polyunsaturated fatty acid [53, 65, 75, 76]. Hepatitis C virus (HCV) also causes massive rearrangements of intracellular membranes leading to the formation of double-membrane vesicles (DMVs) enriched with cholesterol. As was shown in [41], HCV 'usurps' cholesterol transporter proteins, such as NPC1, in order to deliver cholesterol to the viral replication organelle where cholesterol is needed, and blockage of this transporter suppresses the virus replication. Coronaviruses, like Flaviviruses, are assembled and bud from the membranes of Golgi complex and ER [41, 60] and also form double-membrane vesicles [77]. At the same number of newly formed viral particles, the consequences of removing cholesterol from ER membranes by double-membrane vesicles can be more severe than in the case of single membrane vesicles; a quantitative assessment of this process is necessary.

A possible mechanism stimulating the delivery of cholesterol to ER from plasma membrane during coronavirus replication was demonstrated by Wang et al. 2020 [78]. The authors reported that SARS-CoV2 activates the host cell gene encoding cholesterol 25-hydroxylase and induces the formation of 25-hydroxycholesterol, which increases cholesterol availability [79] and triggers its delivery from the plasma membrane to the endoplasmic reticulum, where cholesterol is required for the viral envelope formation. Although, as is known [14, 23–29, 32–41, 46–55], the depletion of cholesterol in the plasma membrane suppresses the virus entry into the cell, the observed trafficking of cholesterol into the ER (normally the flow goes in the opposite direction [71, 72]) can reflect an increased uptake of cholesterol for the formation of envelopes of new viruses. After the release of newly formed viruses, this depleted cell will not be susceptible to new infection.

Thus, the formation and release of viral particles from the cell cannot but affect the composition of the host cell membranes. It can be expected that after a full replication cycle of viruses with high envelope cholesterol, the level of cholesterol in the membranes of the host cell will be reduced, and this depletion of cholesterol can lead to a significant deregulation of cholesterol-dependent processes, including intracellular signaling and metabolic pathways. At a high rate of assembly of a large number of viruses, infected cells may not be able to compensate for the loss of cholesterol in their membranes, and this can lead to cell death due to destabilization and permeabilization of cell membranes. Indeed, ample evidence indicates that lowered membrane cholesterol is associated with altered mechanical properties and increased permeability of the membrane [80–83]. Some viral and bacterial proteins trigger apoptosis through lysosomal membrane permeabilization leading to release of cathepsins [81]. The human immunodeficiency virus type 1 (HIV-1) protein Nef is one of such proteins: when entering mammalian cells, it causes permeabilization of the lysosomal membrane [81]. It seems appropriate at this point to recall that permeabilization of intracellular membranes due to cholesterol depletion underlies the cytotoxic effect of some anticancer drugs [81, 84–87]. As was shown by Appelqvist et al. 2011 [84], the mechanism of action of cisplatin and some other lysosomotropic drugs at least partially is based on the permeabilization of lysosomal membranes leading to cell death; cholesterol accumulation in lysosomal membranes caused by inhibition of cholesterol transporting protein NPC1 prevented the lysosome-dependent cell death [84]. Note that in the case of virus infection, inhibition of the NPC1-dependent cholesterol transport suppressed the virus replication [41] and rescued the infected cells. When NPC1 functions normally and cholesterol is delivered from lysosomal compartment to ER for the formation of viral envelopes, lysosomal membranes lose cholesterol and become leaky; in a way,

virus acts like a lysosomotropic drug. Cholesterol supplementation was also shown to reverse a strong cytotoxic effect on colon cancer cells caused by a low molecular weight compound TASIN-1 producing cholesterol-dependent ER stress triggering oxidative stress and JNK-dependent apoptosis [85].

Thus, virus–cell interactions lead to significant modulations in the lipid composition of cell membranes. A decrease in cholesterol in cell membranes owing to the formation of viral envelopes can be one of the most dangerous consequences of the virus particle assembly, as the amount of cholesterol removed from the cell membranes by newly formed viruses can exceed the compensatory resources of the cell. If the delivery of cholesterol to the cells is insufficient, deregulation of cholesterol-dependent processes can lead to massive cell death, which manifests itself in the clinical course of the disease and a poor prognosis. In this connection, it should be noted that in patients infected with COVID-19, a significant decrease (several fold) in total cholesterol and low-density lipoprotein (LDL) cholesterol levels was recorded [13, 88], and cholesterol-lowering treatments (such as statins) may not be advisable for patients with life-threatening COVID-19 infection, at least until they recover from the infection [88]. Such a drop of the LDL cholesterol level in COVID-19 patients may reflect an enhanced recruitment of circulating cholesterol by the cells to compensate for its loss associated with virus reproduction. Perhaps the clinical prognosis depends on the timely and successful delivery of cholesterol required for cell membrane repair. Another direction in the development of drugs for the treatment of the disease is the search for agents that interfere with the interactions of viral proteins with cholesterol, and this search should be based on an understanding of the mechanisms of these interactions. So far, the only drugs for which clinically significant results have been demonstrated against COVID-19, are dexamethasone and some other corticosteroids [89–91]; secosteroids (vitamin D) are shown to help, too [92]. The use of dexamethasone led to a reduction in mortality to one third of hospitalized patients with severe respiratory complications from COVID-19. It seems possible that the action of steroids may be associated with the repair of a cell membrane damaged by virus-induced depletion of cholesterol.

How does the virus manage to bind and remove cholesterol from cell membranes?

4. Cholesterol recognition/interaction amino acid consensus (CRAC) motifs in viral proteins. Possible uses of CRAC-containing peptides

As was shown earlier [16, 17, 93], some proteins involved in cholesterol-dependent cell functions possess the so-called cholesterol recognition/interaction amino acid consensus (CRAC) motifs – small regions with a specific set of amino acid residues involving a branched apolar aminoacid residue (Val (V), Leu (L), or Ile (I)), aromatic residue (Tyr (Y)), and cationic aminoacid residue (Arg (R) or Lys (K)); these motif-forming amino acids are separated by short segments of any 1–5 amino acid residues. In subsequent discussions, more candidates of aromatic amino acid residues were proposed, and the general formula for the CRAC motifs presumably involved in the interaction of protein with cholesterol presently appears as follows: $V/L/I–X_{(1–5)}–W/Y/F–(X)_{(1–5)}–R/K$, where X stands for any amino acid residue [94–100]. Although the predictive value of this formula has been questioned [95–97], the presence of this motif in many proteins and its participation in the protein–cholesterol interactions has been confirmed by different methods [16, 17, 93–95]. The formula of the CRAC motif can be further developed [94]; important is the very concept of a motif mediating the interactions of cholesterol-dependent proteins with cholesterol.

CRAC motifs are found in many viral proteins, and their role in cholesterol-dependent virus–cell interactions have been demonstrated. For example, CRAC motifs are present in the HIV matrix protein p17, which was shown to participate in virus entry through the raft domains of the cell membranes [27, 101]. The α-helical domain of the hepatitis C virus nonstructural protein NS5A, which is anchored at the cytoplasmic leaflet of the endoplasmic reticulum and is involved in replication hepatitis C virus, also contains CRAC motif [102]. Importantly, peptides derived from this domain were shown to exhibit a broad-spectrum anti-viral activity. Cheng et al. 2008 [103] reported that peptide C5A containing amino acid residues 3–20 of the amphipathic α-helical N-terminal domain of hepatitis A virus protein NS5A suppressed the virus replication by more than 5 orders of magnitude. The authors did not mention the CRAC concept; however, the active peptide C5A (SWLRDIWDWICEVLSDFK) clearly contains the CRAC motif: RDIWDWICEV. Later, the antiviral activity of this peptide C5A against HIV was also demonstrated [104]. It is possible that peptide C5A, owing to the presence of the CRAC motif, binds cholesterol and competes for cholesterol binding with viral protein and thus inhibits the formation of the viral particle.

CRAC motifs are found in alpha-helices of matrix protein M1 of influenza A virus [105–107]. An important role of these CRAC motifs in the organization of the raft structure of the virion membrane was substantiated by using the method of directed point mutations in the CRAC-containing α-helices in the M1 protein [106, 107]. Further studies revealed that M1-derived peptides containing CRAC motifs LEVLMEWLKTR, NNMDKAVKLYRKLK, GLKNDLLENLQAYQKR, corresponding to α-helixes 3 (aa 39–49), 6 (aa 91–105) and 13 (aa 228–243), respectively, to a different extent modulate cholesterol-dependent interactions of cultured macrophages with 2-μm particles that mimic bacteria (phagocytic index). Of the three peptides, NNMDKAVKLYRKLK was most potent and stimulated the cell activity by 50–60% at 35 μM [108]. Peptide RTKLWEMLVELGNMDKAVKLWRKLKR obtained by combining two of these short peptides and containing two CRAC motifs produced much stronger and more complex effect: in a narrow range of low concentrations (1–5 μM) the peptide exerted a stimulatory effect and at 50 μM the peptide was cytotoxic [109]. Reducing the cholesterol content in the cells with methyl-β-cyclodextrin (mβCD) abolished the stimulatory component and lowered the peptide concentration required for the toxic effect. Substitution of the motif-forming amino acids abolished these effects [110]. The cytotoxic effect of the M1-derived peptide RTKLWEMLVELGNMDKAVKLWRKLKR can be explained by the binding (sequestration) of membrane cholesterol by the peptide; this can imitate removal of cholesterol from cell membranes, which occurs during the formation of the viral envelope.

S proteins of coronaviruses SARS-CoV and SARS-CoV2 also contain CRAC motifs [51, 109, 110]. For example, in the case of the S protein of coronavirus SARS-CoV, the CRAC motif YIKWPWYVW is located in the "aromatic" region of the transmembrane domain of the S-protein; this highly conserved region of the S-protein was shown to be necessary for the infection of cells with coronavirus [111, 112].

If the assumption about the essential role of sequestration and removal of membrane cholesterol by viral CRAC-containing proteins in the COVID-19 parthenogenesis is correct, then in order to prevent this destructive action for the cell, it is necessary to maintain a safe level of cholesterol in the plasma membrane. A significant decrease in total cholesterol and low-density lipoprotein (LDL) cholesterol levels in COVID-19 patients [13, 88] may be indicative of the critical loss of cholesterol by cells, and an efficient cholesterol delivery to the cholesterol-depleted cells may be helpful. Cyclodextrins are possible candidates as non-toxic

cholesterol transporters [113–116], which can redistribute cholesterol from endogenous and/or exogenous sources. The use of cyclodextrins increased the lifespan of NPC1$^{-/-}$ experimental mice [117] and improved the condition of patients with Nieman–Pick disease [118]. Another alternative is to prevent viral proteins from interacting with membrane cholesterol. At least some of the drugs that are currently tested – for example, polyphenolic substances like quercetin and saponin glycyrrhizin [119, 120] – can act at the protein/cholesterol interface and hinder cholesterol binding by the CRAC motif of the viral S protein and thus inhibit the assembly of new viral particles. Glycyrrhizin, an active component of liquorice roots, was shown to inhibit SARS-CV replication in Vero cells and replication of SARS-associated coronavirus [121, 122]. However, such agents are not very selective and can affect other cholesterol-dependent proteins and therefore cause side effects. Perhaps specially designed CRAC-peptides specifically blocking the interactions of S-protein with cholesterol will prevent the cellular cholesterol loss leading to permeabilization of membranes, oxidative stress, and cell death. The ability of CRAC-containing peptides to regulate cholesterol-dependent cell functions has been demonstrated in a number of works [17, 109, 122, 123], and studies of the antiviral activity of these peptides may be useful and promising.

5. Conclusions

The SARS-CoV2 pandemic has sparked a brainstorming session over the underlying mechanisms of viral diseases. Many assumptions have been made. This chapter considers possible consequences of cholesterol depletion in the membranes of infected cells due to the formation of cholesterol-rich viral envelopes. At a high viral load and high replication rate the reduction in the cholesterol level in the cell membranes can lead to their permeabilization and subsequent cell death, and this can be one of the factors in pathogenesis of diseases induced by SARS-CoV2. Cholesterol-recognition/interaction (CRAC) motifs in viral proteins may represent a mechanism for the binding of the viral protein with cholesterol. Substances preventing these interactions of viral proteins with cholesterol can suppress the formation of the viral envelope and therefore can be studied as possible antiviral drugs. Peptides containing CRAC motifs from viral proteins may be among these substances.

Acknowledgements

The work was partially supported by the Russian Foundation for Basic Research (project no. 18-04-01363).

Conflict of interest

The author declares that there is no conflict of interest.

Author details

Antonina Dunina-Barkovskaya
Belozersky Institute of Physico-Chemical Biology, Moscow Lomonosov State University, Moscow, Russia

*Address all correspondence to: dunina@belozersky.msu.ru

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Section 2

Hypercholesterolemia in Children

Chapter 6

Familial Hypercholesterolemia

Mariana Suárez Bagnasco

Abstract

Familial hypercholesterolemia is a genetic and metabolic disorder associated with an increased risk of morbidity and mortality. Two main types of familial hypercholesterolemia are distinguished: heterozygous familial hypercholesterolemia and homozygous familial hypercholesterolemia. Homozygous familial hypercholesterolemia progresses much more aggressively with higher levels of LDL-C and higher risk of cardiovascular disease at earlier ages. The prognosis of homozygous familial hypercholesterolemia largely depends on the LDL-C levels. Reducing the LDL-C level is one of the primary goals of treatment patients with familial hypercholesterolemia. Effective control of LDL-C significantly reduces the cardiovascular morbidity and mortality. Understanding the factors likely to affect treatment adherence is paramount. Adherence to treatment can be improve when a genetic etiology is confirmed. Positive genetic test result has beneficial effects on adherence to pharmacotherapy and in achieving LDL-C levels reduction.

Keywords: familial hypercholesterolemia, adherence, illness perception, barriers, diagnose

1. Introduction

This chapter reviews the definition, etiology, course and treatment of familial hypercholesterolemia, and analyses the influence of some factors that may influence the early diagnosis of familial hypercholesterolemia and the treatment of familial hypercholesterolemia.

2. Definition and etiology of familial hypercholesterolemia

Familial hypercholesterolemia (FH) is a genetic and metabolic disorder that affects the metabolism of cholesterol [1–10].

Currently, the genes involved in HF are the following: low-density lipoprotein receptor (LDLR) gene, apolipoprotein B-100 (APOB) gene and proprotein convertase subtilisin kexin type 9 (PCSK9) gene, LDLR adaptor protein 1 (LDLRAP1) gene [1–10]. See **Table 1**.

The mutation in LDLR gene, APOB gene and PCSK9 gene is inherited following an autosomal dominant/autosomal co-dominant pattern and the mutation in LDLRAP1 gene is inherited following an autosomal recessive pattern [1–10].

Mutations of the genes cause defective LDL uptake and degradation, which in-turn leads to an elevation of plasma low-density lipoprotein-cholesterol (LDL-C) level, producing the hypercholesterolemia phenotype. The chronic exposure to high levels of LDL-C lead to the development of atherosclerosis and cardiovascular disease at an early age [1–10].

Gene	Protein	Chromosomal location	Proportion of patients with FH
LDLR	LDL receptor	19p13.2	80–85%
APOB	Apolipoprotein B100	2p24.1	5–10%
PCSK9	Proprotein convertase subtilisin kexin type 9	1p32.3	< 1%
LDLRAP1	LDL receptor adaptor protein 1	1p36.11	< 1%

Table 1.
Genes involved in familial hypercholesterolemia.

Two main types of HF are distinguished: heterozygous familial hypercholesterolemia and homozygous familial hypercholesterolemia. Heterozygous familial hypercholesterolemia is usually caused by a single pathogenic variant in one of the genes associated with familial hypercholesterolemia, mostly in LDLR. Homozygous familial hypercholesterolemia is caused by biallelic pathogenic variants, generally in LDLR [1–10].

3. Diagnosis of familial hypercholesterolemia

Familial hypercholesterolemia often diagnosed using the following diagnostic criteria: UK Simon Broome System and the Dutch Lipid Clinic Network criteria [11–17].

The UK Simon Broome System Criteria can be applied in children/adolescents/adults. The items included in Simon Broome System are the following: laboratory findings (total cholesterol or LDL-C), physical examination (tendon xanthomas), molecular diagnosis (mutation LDLR, APOB or PCSK9), and family history (myocardial infarction, raised total cholesterol) [11–17]. UK Simon Broome System Criteria is attached in **Table 2.**

Items	Criterion
In adults (age ≥ 16): total cholesterol level > 290 mg/dL	A
or LDL-C > 190 mg/dL	
In children (age < 16): total cholesterol level > 260 mg/dL	B
or LDL-C > 155 mg/dL	
Tendon xanthomas in the patient or in a first- or	
second-degree relative	
DNA-based evidence of a mutation in LDLR, APOB, or	C
PCSK9	
Family history of myocardial infarction before age 50	D
in a second-degree relative, or before age 60 in a first degree	
relative	
Total cholesterol >290 mg/dL in a first- or second degree	E
relative	
C or A plus B: Definite familial hypercholesterolemia	
A plus D or A plus E: Probable familial hypercholesterolemia	

Table 2.
UK Simon Broome system criteria.

The Dutch Lipid Clinic Network Criteria can be applied in adults. The items included in Dutch Lipid Clinic Network are the following: laboratory findings (LDL- C), physical examination (tendon xanthomas, arcus cornealis), molecular diagnosis (mutation LDLR, APOB or PCSK9), family history (atherosclerotic cardiovascular disease, tendon xanthomas, arcus cornealis, raised LDL- C), and patient history (coronary artery disease, cerebral or peripheral vascular disease) [11–17]. Dutch Lipid Clinic Network Criteria is attached in **Table 3.**

The diagnostic criteria mentioned differ in the items included and, in the items necessary to make a definitive FH diagnosis. In the Simon Broome criteria, a positive genetic test is sufficient for a definitive diagnosis of familial hypercholesterolemia. In the Dutch Lipid Clinic Network criteria, a positive genetic test should be accompanied by an additional item (for example, elevated LDL-C levels) to fulfill the definite diagnosis criteria [11–17].

Although all the criteria mentioned include LDL- C levels, there are variations in the cut-offs necessary for the diagnosis of familial hypercholesterolemia. It is worth mentioning that untreated LDL-C levels vary across genotypes. For example: levels are highest with two LDLR null alleles, lower with two LDLR defective alleles or two mutant PCSK9 alleles, and lowest with two mutant APOB alleles and in double heterozygotes [18–26].

Regardless of the diagnostic criterion used, before the diagnosis of familial hypercholesterolemia is confirmed, secondary causes of hypercholesterolemia should be excluded such as hypothyroidism, renal disease, nephrotic syndrome, liver disease and diets with extremely elevated saturated fat/cholesterol content.

Items	**Score**
First-degree relative with known premature atherosclerotic cardiovascular disease (age < 55 in men, age < 60 in women) or first-degree relative with LDL-C > 95th percentile	1
First-degree relative with tendon xanthomas or arcus cornealis, or child (under age 18) with LDL-C > 95th percentile	2
Premature coronary artery disease	2
Premature cerebral or peripheral vascular disease	1
Tendon xanthomas	6
Arcus cornealis before age 45	4
LDL-C ≥ 330 mg/dL	8
LDL-C between 250 and 329 mg/dL	5
LDL-C between 190 and 249 mg/dL	3
LDL-C between 155 and 189 mg/dL	1
Functional mutation in the LDLR, APOB, or PCSK9 gene	8
Score > 8 Definite familial hypercholesterolemia	
Score between 6 and 8 Probable familial hypercholesterolemia	
Score between 3 and 5 Possible familial hypercholesterolemia	
Score < 3 Unlikely	

Table 3.
Dutch lipid clinic network criteria.

Furthermore, there are several conditions with overlapping laboratory findings or family history features that might be considered when a diagnosed of familial hypercholesterolemia is suspected. For example: sitosterolaemia (xanthomas and hypercholesterolemia caused by an autosomal recessive pathogenic variant in ABCG5 or ABCG8) and lysosomal acid lipase deficiency (elevated LDL-C levels accompanied by fatty liver disease could be caused by an autosomal recessive pathogenic variant in LIPA) [27–29].

Once an individual is identified with familial hypercholesterolemia (index case) the cascade screening of family members of the known index case is recommend for identify new cases of familial hypercholesterolemia. Cascade screening could include LDL-C measurement, genetic testing, or both [11–17, 30].

Though the diagnosis of familial hypercholesterolemia can be performed without genetic testing (for example, using Simon Broome criteria), when a mutation compatible with familial hypercholesterolemia is identified, genetic testing serves to confirm the diagnosis of FH. Furthermore, genetic testing could provide discrimination, at the molecular genetic level, between homozygous familial hypercholesterolemia and heterozygous familial hypercholesterolemia. Moreover, pre-test and post-test genetic counseling can facilitate patient's interpretation of genetic test results [11–17].

The International Classification of Diseases, 10th Revision, has a specific diagnose criteria for homozygous familial hypercholesterolemia and heterozygous familial hypercholesterolemia (E78.01) [31].

The ICD10 criteria for heterozygous familial hypercholesterolemia are the following: LDL-C $\geq$ 160 mg/dL (4 mmol/L) for children or LDL-C $\geq$ 190 mg/dL (5 mmol/L) for adults and: a first-degree relative who is similarly affected or a first-degree relative with positive genetic testing for an LDL cholesterol-raising defect in LDLR, APOB or PCSK9 [31].

The ICD10 criteria for Homozygous familial hypercholesterolemia are the following: LDL-C $\geq$ 400 mg/dL (10 mmol/L) and: one or both parents with clinically diagnosed familial hypercholesterolemia or one or both parents with positive genetic testing for two identical (homozygous familial hypercholesterolemia) or non-identical (compound or double heterozygous familial hypercholesterolemia) LDL cholesterol-raising gene defects in LDLR, APOB or PCSK9 or one or both parents with autosomal recessive familial hypercholesterolemia [31].

Familial hypercholesterolemia is considered underdiagnosed. During the diagnosis process, some barriers might arise to early diagnosis of familial hypercholesterolemia in patients and relatives. From medical point, for example: physician's knowledge of FH diagnoses and treatment, the lack of a uniform clinical criteria for FH diagnosis, the availability of genetic testing, physician's knowledge about screening methods (selective, opportunistic, universal, cascade) and the identification of probable cases in different health care levels. From patient point, for example: some patients do not want a personal diagnosis to be disclosed to relatives, some parents experience feelings of guilt related to passing their mutation to their children, and many patients interpret their negative genetic test result as meaning their do not have FH hypercholesterolemia or that their FH hypercholesterolemia is not genetic and thus their relatives cannot have FH [1, 32].

Machine learning and deep learning approach could enhance the identification of familial hypercholesterolemia patients using electronic health record data. For example: the FIND FH model. This model recognizes the clinical phenotype for familial hypercholesterolemia and provides the framework for a HIPAA-compliant method to contact these identified individuals with FH [33–36].

4. Course disease and treatment of familial hypercholesterolemia

The signs and symptoms of homozygous familial hypercholesterolemia and heterozygous familial hypercholesterolemia are similar. However, homozygous FH patients have higher levels of LDL-C and higher risk of cardiovascular disease. The disease progresses much more aggressively, the phenotype become clinical manifest earlier, and cardiovascular events occur at earlier ages in homozygous FH patients. Cardiovascular risk factors and lipoprotein(a) levels adversely affect the course of homozygous and heterozygous FH diseases increasing coronary heart disease rates [11–12, 14–16, 37–43].

The prognosis of homozygous familial hypercholesterolemia and heterozygous familial hypercholesterolemia largely depends on the LDL-C levels. Reducing the LDL-C level is one of the primary goals of treatment homozygous and heterozygous FH. Effective control of LDL-C significantly reduces the cardiovascular morbidity and mortality. To improve cardiovascular risk assessment, the use of imaging techniques to detect asymptomatic atherosclerosis is recommended in both homozygous and heterozygous FH [11–12, 14–16, 41–43].

The carotid intima-media thickness is greater and aortic lesions can be seen identified in heterozygous FH patients between 8 to 10 years of age. During adolescence about 25% of the adolescents with heterozygous FH have demonstrable coronary artery calcium. Clinical manifestation of coronary heart disease can be evident in heterozygous FH patients during the third decade of life. Physical manifestations of sustained elevations of LDL-C (tendon xanthomas and corneal arcus) become apparent during adulthood [44–49].

At birth, homozygous familial hypercholesterolemia patients have a $\geq$ 4-fold increase in plasma LDL-C concentrations. Since early in life cholesterol deposits in tendons (xanthomas), in the cornea (corneal arcus) and around the eye (xanthelasma). Furthermore, cholesterol deposits in coronary arteries, carotid arteries, aortic root, and valve. Therefore, coronary heart disease and supravalvular and aortic valve stenosis are possible causes of death. Young adults with homozygous familial hypercholesterolemia often require aortic valve replacement. Non-invasive imaging can be used to monitor atherosclerotic and aortic valve disease progression in homozygous FH patients and to adjusted treatment [50–55].

Treatment of FH is long-term and involves pharmacotherapy, lifestyle modifications and control other cardiovascular risk factors such as hypertension, diabetes, tobacco smoking, obesity, and sedentary behavior [12, 14–16, 41–42, 56–61].

Statins are the mainstay pharmacotherapy. However, if maximal tolerated dose of statin is used and LDL-C goal not achieved, statins usually combined with ezetimibe. Additionally, if using statin-ezetimibe combination LDL-C goal not achieved, adding PCSK9 inhibitors is considered [12, 14–16, 41–42, 56–65]. The European Atherosclerosis Society/European Society of Cardiology plasma low-density lipoprotein-cholesterol goals [57] for patients with familial hypercholesterolemia are summarized in **Table 4**.

Patients with PCSK9 mutations are particularly responsive to PCSK9 inhibition. However, PCSK9 inhibitors had no effect on LDL cholesterol in those with two LDLR null alleles with homozygous familial hypercholesterolemia. Moreover, if at least one allele had residual LDLR activity, PCSK9 inhibitors lowered LDL cholesterol in patients with homozygous familial hypercholesterolemia [66–69].

Incomplete/low adherence to treatment is associated with increased risk of cardiovascular disease. A proportion of FH patients fall short of full compliance or follow regimens inconsistently. Understanding the factors likely to affect treatment adherence is paramount [70–79].

The European Atherosclerosis Society/European Society of Cardiology (2019) recommends the following goals for plasma low-density lipoprotein-cholesterol for patients with familial hypercholesterolemia:
LDL-C < 3.5 mmol/L in children
LDL-C < 1.8 mmol/L and a reduction in plasma LDL-C of >50% in subjects without other major risk factors (high risk)
LDL <1.4 mmol/L and a reduction in plasma LDL-C > 50% in subjects with one or more major cardiovascular disease (CVD) risk factors and/or existing CVD (very high risk)

Table 4.
LDL-C goals for patients with familial hypercholesterolemia.

As well as in other chronic pathologies that require long-term treatment, psychological and cognitive issues can influence adherence to treatment [70–79].

While there is no evidence of depression or anxiety in FH patients, instead there is evidence of cognitive deficits and mild cognitive impairment in FH patients. Deficits in executive functioning and memory may affect medication adherence because taking medicines involves developing and implementing a plan to adhere and remembering the plan (for example: the plan may require time-based (e.g., at 8:00 p.m.) or event-based prospective remembering (e.g., with meals) and remembering what medicine take and whether the medicine was taken). Furthermore, executive functions may affect the achievement of lifestyle modifications and maintain healthy behavior over time included in FH management [70–76].

Ilness perceptions may affect adherence to both lifestyle interventions and medications. Perception of illness/perception of risk may affect FH patient behavior. Risk perception may be changed by personal or familiar events, such as a cardiovascular event in the family, a change in or an onset of symptoms and becoming parent. Health staff need to recognize variation in patient's risk perception because it can affect medical treatment [77–79].

Adherence to FH treatment can be improve when a genetic etiology is confirmed. Positive genetic test result has beneficial effects on adherence to pharmacotherapy and in achieving LDL-C levels reduction. Patients whose diagnosis was confirmed by genetic testing perceived diagnosis more accurate, believed more strongly that genes controlled their cholesterol and have higher perceived efficacy of medication. In children with FH, parents are critical in promoting treatment adherence [77, 80–85].

5. Conclusions

Although the diagnosis of familial hypercholesterolemia can be performed without genetic testing, knowledge about the genetic status of an individual with familial hypercholesterolemia can improve understand of risk and prognosis as well as improve managing familial hypercholesterolemia. Adherence to FH treatment can be improve when a genetic etiology is confirmed.

Conflict of interest

The authors declare no conflict of interest.

Author details

Mariana Suárez Bagnasco
Catholic University of Uruguay, Montevideo, Uruguay

*Address all correspondence to: mariansb@gmail.com

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Chapter 7

Arterial Stiffness Assessment in Children with Familial Hypercholesterolemia

Dinara Sadykova, Liliia Galimova, Evgeniia Slastnikova, Zulfiia Khabibrakhmanova and Natalya Guseva

Abstract

Familial hypercholesterolemia (FH) is the genetic disease which characterized by an increase of level total cholesterol and low density lipoproteins since childhood. The aim of the study was to assess arterial stiffness in children with heterozygous FH by measuring the pulse wave velocity (PWV) in the aorta. The study involved 118 children, 60 healthy children in the control group and 58 children with heterozygous FH in the main group. Both groups were divided into 3 age subgroups: 5–7 years old, 8–12 years old and 13–17 years old. The diagnosis of FH was made using British criteria by Simon Broome. The lipid profile was determined for all children, blood pressure was monitored daily with an estimate of the minimum, mean and maximum PWV (PWVmin, mean PWV, PWVmax) in aorta using oscillometric method. Correlation analysis in patients with FH revealed direct correlation between PWVmin, mean PWV and PWVmax with total cholesterol ($r = 0.46$, $r = 0.46$ and $r = 0.464$, respectively, $p < 0.001$). The study demonstrates an increase in the PWV in the aorta in children with FH compared with healthy peers from 8–12 years of age and a progression of arterial stiffness most significant in the group of 13–17 years.

Keywords: familial hypercholesterolemia, arterial stiffness, pulse wave velocity, children

1. Introduction

Familial hypercholesterolemia (FH) is a monogenic disease with a predominantly autosomal dominant mode of inheritance, accompanied by a significant increase of the low-density lipoprotein cholesterol (LDL-C) level in the blood [1]. Patients with FH have a high risk of early development of atherosclerosis [2, 3]. The incidence of FH in the general population is estimated at about 1 per 200 persons [3], in addition, the evidence exist that in patients with established coronary heart disease, the prevalence of potential FH is up to 8.3% in men and 11.1% in women [4]. However, despite this, there is a low level of diagnosis and treatment [5, 6]. In this regard, registers of patients with FH have recently been developed for assessing the level of diagnosis, treatment and improvement in results of therapy [7, 8]. In

more than 90% of cases, FH is caused by mutations in the gene encoding the LDL receptor, which reduce the cellular uptake of LDL and, therefore, significantly increase their plasma level [9]. Mutations in other genes leading to the same phenotype have also been identified: associated with apolipoprotein B, which affect the LDL-binding domain of apolipoprotein B as the most important apolipoprotein for uptake of LDL particles, and mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9) [10, 11].

It is known that high plasma cholesterol level is a risk factor for the development of cardiovascular diseases [12] and may be the cause of early vascular damage. Currently, there are methods that allow registering pathological changes in blood vessels at preclinical stage. The detection of early changes in the walls of arteries by non-invasive methods, such as ultrasound duplex scanning, assessment of central aortic pressure and pulse wave velocity, has opened up new perspectives, helping to identify high-risk patients [13–17].

Several studies have shown that hypercholesterolemia can cause the loss of elasticity and increased stiffness of arterial vessels, leading to an increase in the pulse wave velocity due to its rapid spreading in stiff arteries [18, 19]. Arterial stiffness is considered to be a significant predictor of overall and cardiovascular mortality in patients with arterial hypertension [20–23], in patients with end-stage renal failure [24, 25] and in the elderly patients [26]. In addition, it was noted that arterial stiffness is closely related to structural changes in the artery such as thickening of the intima-media complex [27]. Changes in the elastic properties of arteries may indicate a functional disorder long before the appearance of clinical symptoms. One of the indicators for assessing the stiffness of the arteries is the pulse wave velocity (PWV) measurement in aorta. It is the measurement of the speed of pulse pressure propagation along a segment of the arterial vessels [28]. It should be noted that the measurement of the arterial stiffness is quite widespread among adult patients [29, 30], while in pediatrics, despite its non-invasiveness and high informativeness, it is used much less frequently. This is probably due to the complexity of standardization, time costs and the need for additional equipment.

2. The aim of this study

The aim of this study is to assess arterial stiffness in children with heterozygous familial hypercholesterolemia by measuring the pulse wave velocity in the aorta

3. Materials and methods

The study involved 118 children. The control group consisted of 60 healthy children and 58 children with heterozygous familial hypercholesterolemia formed the main group (**Table 1**). The diagnosis of FHC was established in accordance with the British Simon Broome criteria [31]. The study included children with FH who were not taking statins. 15 patients from the main group underwent genetic testing in the Health in Code laboratory (Spain) for the detection of a monogenic mutation responsible for the development of familial hypercholesterolemia, and a positive DNA test was obtained. Exclusion criteria: secondary dyslipidemia, arterial hypertension, obesity. Written informed consent was obtained from all the participants of the study.

	Control group, n = 60	**Main group, n = 58**
Age, years (M ± σ)	11,53 ± 4,2	10,92 ± 4,1
Gender, m/f	40/20	37/21
Smoking, n/%	0(0)	0(0)
Obesity, n/%	0(0)	0(0)
Arterial hypertension, n/%	0(0)	0(0)
Cutaneous xanthomas	0(0)	0(0)
Corneal arch	0(0)	0(0)
Thickening of the Achilles tendon	0(0)	0(0)
TC, mmol/l (M ± σ)	3,5 ± 1,2	7,8 ± 2,3
LDL, mmol/l (M ± σ)	1,6 ± 0,8	6,1 ± 1,2
HDL, mmol/l (M ± σ)	0,9 ± 0,1	1,1 ± 0,3
TG, mmol/l (M ± σ)	0,8 ± 0.4	1,2 ± 0,3

Abbreviations: TC - total cholesterol; LDL - low density lipoprotein; HDL - high density lipoprotein; TG - triglycerides.

Table 1.
Clinical and laboratory characteristics of children of the main and control groups.

Total cholesterol, triglycerides, LDL, and high-density lipoprotein cholesterol (HDL) were measured using commercial kits (Beckman Coulter, USA) on an automatic biochemical analyzer (Au5800 Beckman Coulter, USA).

The clinical examination included a careful life history and family history taking, physical examination, and body mass index (BMI) assessment. All children underwent 24-hour blood pressure monitoring with an assessment of the pulse wave velocity in the aorta using the oscillometric method of the BPLab Vasotens system (Petr Telegin Ltd., Russia).

In the BPLab program for determining PWV in aorta the following ratio is used: PWVaorta = K × (2 × L) /RWTT, where PWVaorta is PWV in the aorta; K is the scale factor for standardizing the obtained value of the PWV; L is the length of the aortic trunk (in BPLab software, the distance from the upper edge of the sternum to the pubic bone is taken as the length of the aorta); RWTT (reflected wave transit time) [32].

4. Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics v.23 software (developed by IBM Corporation, USA). The results were subjected to statistical processing using nonparametric methods in connection with the established absence of a normal distribution of quantitative indicators (testing for normal distribution was carried out using the Shapiro–Wilk test). Quantitative data were described using the values of the median (Me) and the lower and upper quartiles [Q1-Q3]. Comparison of quantitative indicators between two groups was carried out using the Mann–Whitney test, between the three groups using the Kruskal-Wallis test with an a posteriori Dunn test. Correlation analysis was carried out using the Spearman's rank correlation coefficient; the assessment of the tightness of correlation was carried out using the Chaddock scale. Differences in indicators and identified relationships were considered statistically significant at $p < 0.05$.

5. Results

Analysis of the values of the minimum PWV (PWVmin), mean PWV (PWVmean) and maximum PWV (PWVmax) obtained during 24-hour blood pressure monitoring revealed statistically significant differences between the main and control groups ($p < 0.001$) (**Figure 1**). The presence of FHC was accompanied by a significant increase in PWV - minimum, mean and maximum values.

Taking into account the results obtained, we analyzed the degree of change in PWV depending on the age of children. For the analysis, both groups were divided into 3 age subgroups: from 5 to 7 years old, from 8 to 12 years old, from 13 to 17 years old (**Table 2**).

In accordance with the results obtained, in the younger age subgroup (5–7 years old), there were no statistically significant differences in PWV between the children of the main and control groups. In children aged 8–12 years, there was no statistically significant difference in the values of PWV min and mean PWV. While PWVmax was characterized by statistically significantly higher values in the main group (5.1 [4.7–5.8] m/s) relative to the control (4.6 [4.45–5.05] m/s) ($p = 0.041$). The most pronounced changes were found in the group of children with FHC at the age of 13–17 years. In this group were revealed statistically significant differences in the minimum, mean and maximum pulse wave velocity.

Taking into account the peculiarities of physical parameters and age periodization, we have analyzed the dynamics of changes in PWV depending on the age of children. In children of the control group, PWVmin in children 8–12 year old was statistically significantly higher than in children 5–7 year old (3.6 [3.2–4.1] m/s and 3.0 [2.8–3.1] m/s, respectively, $p = 0.049$). When comparing PWVmin in groups of 8–12 years old children and 13–17 years old children, no statistically significant difference was found (3.6 [3.2–4.1] m/s and 3.9 [3.5–4.1] m/s, respectively, $p = 0.052$). A similar dynamics of growth was observed for the mean pulse wave velocity in healthy children when compared in age subgroups 8–12 years old and 5–7 years old (4.3 [3.7–5.1] m/s and 3.8 [3.7–3.9] m/s, respectively, $p = 0.026$) and 8–12 years old and 13–17 years old (4.3 [3.7–5.1] m/s and 4.5 [4.2–4, 9] m/s, respectively, $p = 0.114$).

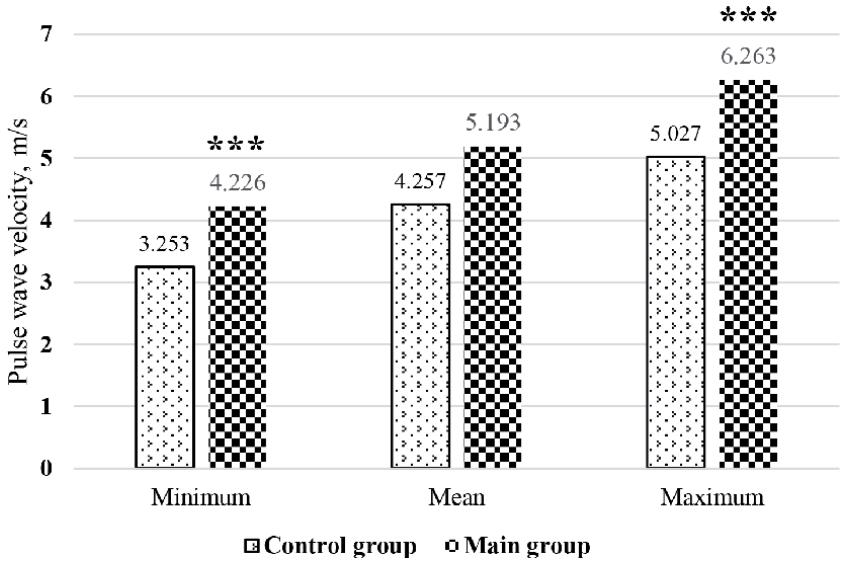

Figure 1.
*Comparison of the values of the pulse wave velocity in the main and control groups. Note: *** - p < 0.001.*

Parameter m/s	Control group		Main group		p
	Me	Q_1-Q_3	Me	Q_1-Q_3	
5–7 years (n = 15)			5–7 years (n = 16)		
PWVmin	3,0	2,8-3,1	3,05	2,6-3,3	0,19
PWVmean	3,8	3,7-3,9	4,1	3,8-4,1	0,19
PWVmax	4,5	4,3-4,8	4,8	4,1-5,3	0,095
8–12 years (n = 22)			8–12 years (n = 21)		
PWVmin	3,6	3,2-4,1	3,5	3,3-3,9	0,052
PWVmean	4,3	3,7-5,1	4,6	4,0-5,0	0,05
PWVmax	4,6	4,45-5,05	5,1	4,7-5,8	0,041
13–17 years (n = 21)			13–17 years (n = 23)		
PWVmin	3,9	3,5-4,1	4,7	4,1-5,1	0,009
PWVmean	4,5	4,2-4,9	5,5	4,8-6,4	0,009
PWVmax	5,4	5,05-5,6	6,2	5,7-7,55	0,007

Table 2.
Comparison of the values of the pulse wave velocity depending on the age of the children.

The analysis of PWVmax showed that these indicators in the group of 5–7 years old and 8–12 years old children did not significantly differ from each other (4.5 [4.3–4.8] m/s and 4.6 [4.45–5.05] m/s, p = 0.145), while in children 13–17 years old it was significantly higher in comparison with 8–12 years old children (5.4 [5.05–5.6] m/s and 4.6 [4, 45–5.05] m/s, respectively, p = 0.022). It should be noted that there is a statistically significant difference in the values of PWVmin, mean PWV and PWVmax in children 13–17 years old relatively to 5–7 years old (**Table 3**).

The study of PWV in children of the main group revealed a statistically significant difference in the increase in PWVmin, mean PWV and PWVmax indicators when compared in all main groups (**Table 4**).

Parameter, m/s	5–7 years	8–12 years	13-17 years	P_{1-2}	P_{2-3}	P_{1-3}
PWVmin	3,0 [2,8-3,1]	3,6 [3,2-4,1]	3,9 [3,5-4,1]	**0,049**	0,052	**0,043**
PWVmean	3,8 [3,7-3,9]	4,3 [3,7-5,1]	4,5[4,2-4,9]	**0,026**	0,114	**0,047**
PWVmax	4,5 [4,3-4,8]	4,6 [4,45-5,05]	5,4 [5,05-5,6]	0,145	**0,022**	**0,018**

Note: p is the level of significance of the differences.

Table 3.
Indicators of the pulse wave velocity of children of the control group.

Parameter, m/s	5–7 years	8–12 years	13–17 years	P_{1-2}	P_{2-3}	P_{1-3}
PWVmin	3,05[2,6-3,3]	3,5 [3,3-3,9]	4,7 [4,1-5,1]	**0,001**	**0,004**	**0,008**
PWVmean	4,1 [3,8-4,1]	4,6 [4,0-5,0]	5,5[4,8-6,4]	**0,001**	**0,016**	**0,004**
PWVmax	4,8 [4,1-5,3]	5,1 [4,7-5,8]	6,2 [5,7-7,55]	**0,028**	**0,021**	**0,018**

Note: p is the level of significance of the differences.

Table 4.
Indicators of the pulse wave velocity of children of the main group.

At the same time, a more significant dynamics of increase in PWV was observed in children with FH compared with the control group in the age range of 13–17 years (**Figure 2**). It should be noted that the dynamics of the increase in indicators in the control group was less than in children with FH (**Figure 3**).

Taking into account the presence of dyslipidemia in patients with FH in the form of severe hypercholesterolemia, we performed a correlation analysis of the relationship between PWV values and lipid metabolism indicators. In the main group, statistically significant direct correlations were established between PWVmin, mean PWV and PWVmax with total cholesterol level (r_{xy} = 0.46 [95% CI: 0.227–0.644], r_{xy} = 0.46 [95% CI: 0.229–0.642] and r_{xy} = 0.464 [95% CI: 0.234–0.645], respectively, $p < 0.001$ in all cases).

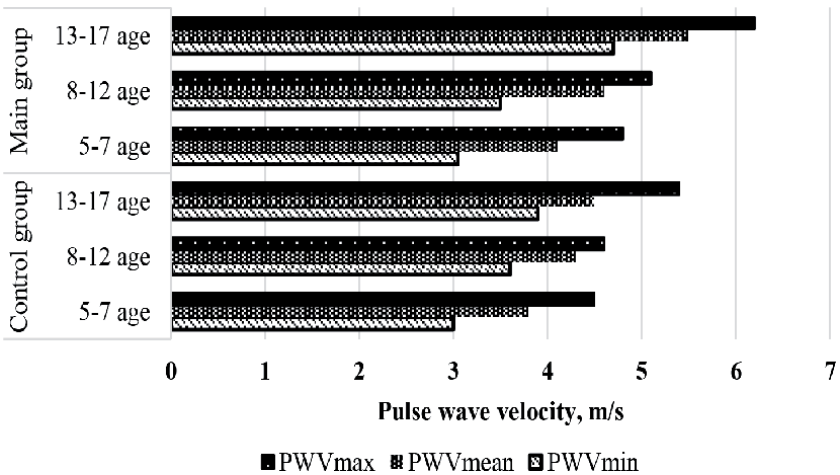

Figure 2.
Indicators of the pulse wave velocity of the main and control groups, depending on the age of the children. Note: PWVmin - minimum pulse wave velocity, PWVmean - mean pulse wave velocity, PWVmax - maximum pulse wave velocity.

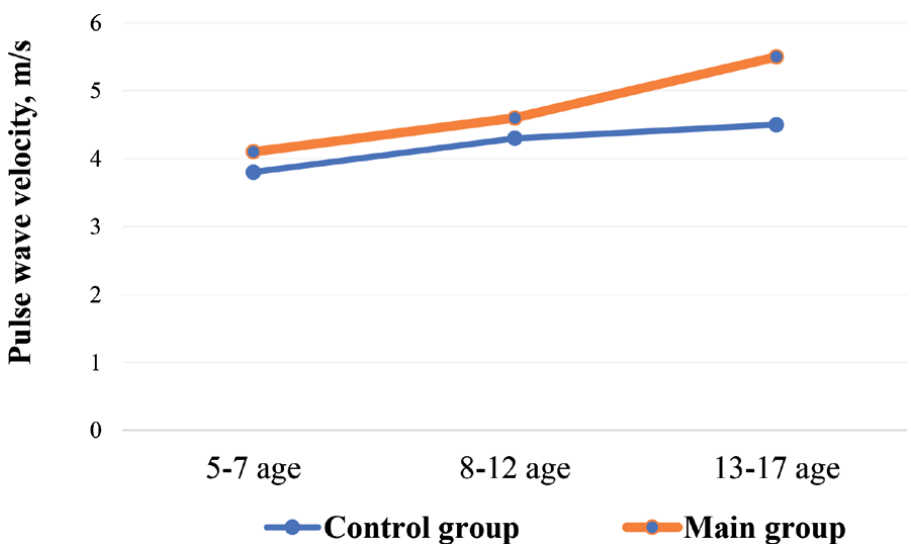

Figure 3.
*Dynamics of the increase in the mean pulse wave velocity in the main and control groups, depending on the age of the children. Note: * - p = 0.009.*

6. Discussion

In the presented study, the condition of the arterial wall was assessed by measuring the pulse wave velocity in the aorta in children with FH and healthy children of the same age. The study design differs from previous studies of PWV in the pediatric population presented in the literature [33–36] in distribution of participants into age subgroups. In our opinion, such an analysis of the PWV values increases the correctness and statistical significance of the obtained results.

We found that the PWV values of children of the main group at the age of 5–7 years do not differ from those of the control group. In the 8–12-year-old subgroup in patients with FH, only the maximum PWV indicators were statistically significantly higher than in the comparison group. The identified deviations probably reflect the initial changes in the stiffness of the vascular wall in children of the main group. The most pronounced differences in the studied parameters were observed in children with FH in the age subgroup of 13–17 years old and were characterized by a statistically significant increase in PWVmin, mean PWV and PWmax relatively to the control group.

In our study, we also analyzed the dynamics of PWV growth depending on age. It was shown that PWV values increase with the age of the child both in familial hypercholesterolemia and in healthy children. This indicates that the studied indicators cannot be the same for all children and it is necessary to use age reference values in pediatrics. In addition, the increase in all three values of PWV was most pronounced in the group of 13–17 year old patients with FH, it allows us to suggest that they have a more pronounced change of properties of the vascular wall already at preclinical stages than in individuals with normal blood lipid profile.

A number of studies have revealed an increased PWV in children with hypertension [37], increased body mass index [38, 39]. In the present study, special attention was paid to patients with FH without risk factors such as smoking, obesity, and high blood pressure. Thus, the authors were able to assess the effect of hypercholesterolemia on the change in PWV precisely. The established correlation between total cholesterol level and PWV values allows us to regard an increase in total cholesterol level as a leading factor in forming the arterial stiffness in children with FH. In addition, the registration of the initial changes in PWV in the group with FH from 8–12 years old with further progression of the process in the absence of such changes in children 5–7 years old indicates a possible cumulative effect of cholesterol and its effect on the artery wall condition. This is consistent with large randomized studies showing that the effect of LDL on the development of atherosclerotic vascular disease is determined not only by the absolute level of LDL, but also by its cumulative effect on target organs [2, 40, 41].

7. Conclusion

Thus, the relationship between cholesterol level, age, and arterial stiffness indicators in familial hypercholesterolemia makes it possible to recommend the study of pulse wave velocity as a possible additional method for studying the cardiovascular risk in children with familial hypercholesterolemia and assessing the progression of the disease

Conflict of interest

Authors declare no conflict of interest.

Acknowledgements

We want to thank the Head of Tatarstan Republic's Ministry of Health, the Rector of Kazan State medical University, Kazan, Russian Federation, the Head of the Children's Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan, Russian Federation for the help in the organization of Children's Lipid Center, where we run our observation of children with Familial Hypercholesterolemia.

Author details

Dinara Sadykova[1,2]*, Liliia Galimova[2], Evgeniia Slastnikova[2], Zulfiia Khabibrakhmanova[1] and Natalya Guseva[2]

1 Kazan State Medical University, Kazan, Russian Federation

2 Kazan State Medical University, Children's Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan, Kazan, Russian Federation

*Address all correspondence to: sadykovadi@mail.ru

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Chapter 8

Dyslipidaemia in African Children and Adolescents

Bose Etaniamhe Orimadegun

Abstract

Dyslipidaemia tends to occur in children and adolescents and steadily worsens through to adulthood. The abnormal lipid profile in children with this disease is like what we see in adults with premature cardiovascular disease (CVD). Identifying children with dyslipidaemia and successfully improving their lipid profile may reduce their risk of accelerated atherosclerosis and premature CVD. In those children with severe dyslipidaemia due to a family history, treatment is used to decrease the risk of cardiogenic events. Screening for lipid disorders in children is based on the rationale that early identification and control of paediatric dyslipidaemia will reduce the risk and severity of cardiovascular complications in adulthood. Though lipid disorders and associated diseases are rare in children in Africa, there has been little research in this field. Emerging research indicates that obesity and cholesterol concerns is on the rise within children and adolescents of African descent. The definition of paediatric dyslipidaemia and the approach to screening, and diagnosis of lipid disorders in children are discussed in this chapter.

Keywords: hypercholesterolemia, lipid disorders, cardiovascular risk, dyslipidaemia, lipoprotein cholesterol

1. Introduction

Overweight and obesity are proven cardiovascular risk factors for both adults and children [1]. These conditions are associated with increasing risk of dyslipidaemia [2, 3]. Unfortunately, the world has experienced a huge increase in obesity with a parallel increase in the risk factor for cardiometabolic disease characterised by insulin resistance, dyslipidaemia, and hypertension, known together as metabolic syndrome [4]. These conditions were previously unheard of in children and adolescents but are now documented in the literature [5]. Current evidence has shown that atherosclerosis, predominantly adult diseases marked by the accumulation of fatty material on the inner wall of the arteries, starts in childhood as an alteration of lipid concentration and can be related through puberty to modifications that contribute to the development of this disorder. Children and adolescents with elevated cholesterol levels are more likely to experience dyslipidaemia in adults than their counterparts in the same population [6, 7]. Identification of dyslipidaemia is therefore essential for the prevention or cessation of atherosclerotic processes during childhood and ultimately for the prevention of premature cardiovascular disease.

Lipid disorders and related diseases are rare in children and adolescents in Africa and there is a scarcity of literature on this topic. However, emerging data indicates

that the incidence and prevalence of obesity and dyslipidaemia is on the rise in the population of African children and adolescents, partially due to shifts in economic and lifestyle towards the trends in the Western world [8]. Serious comorbidities, complications, and cardiovascular risk factors, including obesity, diabetes mellitus, hypertension, and smoking, are correlated with dyslipidaemia. As a result, more attention tends to be paid to the increasing problems of dyslipidaemia among the African population in recent years. The key objectives of this chapter are to discuss the burden of dyslipidaemia, diagnosis, risk factors and health problems, as well as gaps in awareness of dyslipidaemia in children and adolescents in Africa.

2. What is dyslipidaemia in children?

From a general biology perspective, lipids are organic and water insoluble compounds which include fatty acids, triglycerides, and cholesterol. Lipoproteins are also soluble in watery environments of human body. Chylomicrons are formed in the intestine after fat is digested. They are then moved to the fat tissue, muscle, and liver. Chylomicrons are hydrolysed into free fatty acids and then metabolised to low density lipoprotein cholesterol (LDL-C) (the major carrier of cholesterol to tissues). Cholesterol is a fatty substance that passes through high density lipoprotein cholesterol (HDL-C) to peripheral tissues and then to the liver. Abnormalities in the pathway lead to dyslipidaemia.

Dyslipidaemias are lipoprotein metabolism disorders that result in the abnormalities of high total cholesterol (TC), high LDL-C, high non-HDL-C, high triglycerides, and low HDL-C. The HDL and LDL cholesterol monitor the amount of cholesterol that can occur in the body, and if there is an excess it can increase the risk of cardiovascular events. Other forms of dyslipidaemia also include high phospholipids and combined dyslipidaemia.

Since cholesterol is an essential component of human cells, cholesterol may also be generated by individual cells or introduced to the body via our diets. However, when cholesterol levels are increased for whatever reason, they may be bad for the human body. Lipid levels in children younger than 19 years of age are different from lipid levels in adults and vary for the same age in different patients. As an infant, the levels of cholesterol and triglycerides are lower than when a person is an adult. Levels grow steadily during the first year of adolescence, then increase more slowly until they reach the age of 9 to 11 years, but then increase slightly faster until they reach adulthood. At puberty, low-density lipoprotein cholesterol (LDL-C) blood levels decrease by about 10% to 20% or more, whereas high-density lipoprotein (HDL-C) levels increase by 50% or more.

The plasma levels of serum lipids and lipoproteins as recommended are in **Table 1**. Normative data are used to establish cut-off points and identify ranges of acceptable, borderline, and abnormal levels as shown. In **Table 1**, the values for plasma lipid and lipoprotein levels are taken from the National Cholesterol Education Program's (NCEP) Expert Panel on Cholesterol Levels in Children as they were observed. Non-HDL-C values from the Bogalusa Heart Study are equivalent to the NCEP Paediatric Panel cut-off points for LDL-C. Values for plasma Apo B and Apo A-1 come from the National Health and Nutrition Examination Survey III (NHANES III). As a usually occurring wide range, the threshold points for high and borderline-high values reflect roughly the 95th and 75th percentiles, respectively. These values fall into the range of the 10th percentile of the standard range for HDL-C and ApoA-1. It should be noted that the ranges for plasma lipoprotein cholesterol in **Table 1** are consistent with the guidelines of the National Heart, Lung and Blood Institute, the American Academy of Paediatrics and the American

Category	Acceptable mg/dL (mmol/L)	Borderline mg/dL (mmol/L)	High mg/dL (mmol/L)
TC	<170 (4.4)	170 to 199 (4.4 to 5.2)	≥200 (5.2)
LDL-C	<110 (2.8)	110 to 129 (2.8 to 3.3)	≥130 (3.4)
Non-HDL-C	<120 (3.1)	120 to 144 (3.1 to 3.7)	≥145 (3.8)
ApoB	<90 (2.3)	90 to 109 (2.3 to 2.8)	≥110 (2.8)
TG			
0 to 9 years	<75 (0.8)	75 to 99 (0.8 to 1.1)	≥100 (1.1)
10 to 19 years	<90 (1 mmol/L)	90 to 129 (1 to 1.5)	≥130 (1.5)
HDL-C	>45 (1.2)	40 to 45 (1 to 1.2)	<40 (1)
ApoA-1	>120 (3.1)	115 to 120 (3 to 3.1)	<115 (3)

Adapted from expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents, national heart, lung, and blood institute [3].

Table 1.
Acceptable, borderline-high, and high plasma lipid and lipoprotein ranges for children and adolescents.

College of Cardiology. However, these cut-off points have not been validated as accurate benchmarks for accelerated atherosclerosis and CVD events in the African children's population.

3. Problem of dyslipidaemia in children

Atherosclerosis and cardiovascular disease (CVD) are the major health problems associated with dyslipidaemia. These disorders are vascular problems associated with more than 17 million deaths worldwide in 2015, a rise of 12.5 per cent from 2005 onwards [9]. While it is acknowledged that a diet low in saturated fat and regulated cholesterol levels are essential for heart health, it is also determined that certain foods can increase the risk of coronary artery disease (CAD) and other cardiovascular problems [10–12]. Although the prevalence of dyslipidaemia has gradually decreased in several high-income and developing countries over the last 20 years, it is currently predicted that the incidence of dyslipidaemia will increase in African countries due to the rapid change in lifestyles to high-income and developed countries [13].

As far back as 1981, evidence from different studies among Caucasians showed that in childhood, serum levels of cholesterol and triglycerides could rise to levels similar to those seen in young adults at around 2 years of age [14]. Concentrations and turnover of such important molecules in blood lipid concentrations do occur in children. Over the years, researchers have found that if there is a family history of CVD, there is greater concern that a CVD will be developed.

There is ample evidence which suggests that there are more children and adolescents with the hyperlipidemia disease. From the 1988–1994 National Health and Nutrition Survey, it was shown that 10 percent of teenagers had the total cholesterol greater than 200 mg per dL [15]. Also, the newly generated age- and gender-specific lipoprotein from data of the Child and Adolescent Trial for Cardiovascular Health showed that over one-tenth of children aged 9 to 10 years had TC levels greater than 200 mg per dL [16].

While data on the severity of dyslipidaemia among children and adolescents in Africa are scarce in published literature, a few observational studies have reported hypercholesterolemia prevalence and associated risk factors. In the Ghana School Survey conducted in two cities, Kumasi and Accra, the proportion of children with

hyperlipidemia was 12.1% for TC, 4.5% for TG, 28.4% for HDL-C and 9.2% for LDL-C [17]. Another study conducted among adolescent school children in the Eti-Osa Local Government Area of Lagos State, Nigeria, recorded that only 3.6 per cent of participants had TC greater than 200 mg/dL [18]. The highest prevalence of high TC among Angolan pre-pubertal adolescents, 7 to 11 years of age, was estimated to be 69.2% [19].

4. Causes of dyslipidaemia

Dyslipidaemias in children and adolescents can be inherited and/or acquired. Acquired causes of dyslipidaemia can be nutritional or secondary to other diseases. Excessive dietary intake of saturated and trans fats can be a major cause of dyslipidaemia. Hereditary types are referred to as primary dyslipidaemias which includes monogenetic and polygenic defects.

In the clinical setting, a primary metabolic disorder indicates that there is a deficiency in the lipid metabolism, and this is designated familial hypercholesterolemia (FH). The FH follows an autosomal dominant pattern of inheritance and is characterised by an increase of high TC and LDL-C since birth. Earlier onset of atherosclerotic cardiovascular disease (ASCVD) is also seen [20, 21]. Studies show that FH arises from genetic mutations in the LDL receptor (LDLR) and from the action of proprotein convertase known as proprotein convertase 9 (PCSK9) when it mutates [22, 23]. Also, FH has been associated with mutations in the apolipoprotein B gene which impedes the binding of LDL particles to the LDL receptor gene [22, 23].

Exogenous causes	**Hepatic causes**
• Alcohol • Drug therapy including corticosteroids, isotretinoin, some oral contraceptives, select chemotherapeutic agents, and select antiretroviral agents	• Obstructive liver disease/cholestatic conditions • Biliary cirrhosis • Alagille syndrome
Endocrine/Metabolic causes	**Inflammatory disease**
• Hypothyroidism/hypopituitarism	• Systemic lupus erythematosus
• Diabetes mellitus types 1 and 2	• Juvenile rheumatoid arthritis
• Pregnancy	**Storage disease**
• Polycystic ovary syndrome	Glycogen storage disease
• Lipodystrophy	• Gaucher disease
• Acute intermittent porphyria	• Cystine storage disease
Renal causes	• Juvenile Tay-Sachs disease
• Chronic renal disease	• Niemann-Pick disease
• Haemolytic uremic syndrome	**Other causes**
• Nephrotic syndrome	• Kawasaki disease
Infectious causes	• Anorexia nervosa
• Acute viral/bacterial infection	• Childhood cancer survivor
• HIV infection	• Idiopathic hypercalcemia
• Hepatitis	• Klinefelter syndrome

Adapted from expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents, national heart, lung, and blood institute [3].

Table 2.
Causes of secondary dyslipidaemia in children and adolescents.

To date, reports of FH cases in Africa have been rare. There may be several explanations for this. Perhaps, the gene implicated in the aetiology of FH is rare in African population. Even though cardiovascular diseases affect many Africans, it is still important for more studies to be performed on genetic factors that can cause dyslipidaemia. This would reduce the burden of cardiovascular disease in Africa.

Secondary causes of dyslipidaemia are due to "non-lipid" underlying conditions rather than an inborn lipid metabolism disorder, some of which are shown in **Table 2** [24].

5. Screening for dyslipidaemia in children

Usually, screening tests are performed on people who do not show any symptoms of disease to detect health problems or illnesses. The key goal of screening is early detection, to reduce the risk of illness, or to identify it early enough to treat it more effectively. Childhood lipid disorder screening is focused on the rationale for reducing the risk and severity of cardiovascular disease (CVD) in adulthood by early detection and management of paediatric dyslipidaemia. Universal screening for dyslipidaemia became the recommended practice in 2011 [25]. Currently, in most parts of Africa, screening for hyperlipidemia in children and adolescents is not routinely performed in clinical settings. However, it is appropriate to become aware of the latest guidelines for the diagnosis and treatment of dyslipidaemia by child health practitioners. The National Cholesterol Education Program Expert Panel on Blood Cholesterol in Children and Adolescents issued the first guidelines for paediatric lipid screening in 1992 [26]. By 2011, when the American Heart Association [27] and the American Academy of Paediatrics included parameters to identify high and moderate risk individuals as seen in **Table 3**, thereafter, the guideline developed over several years with modifications [29].

The findings of National Heart Lung and Blood Institute (NHLBI) panel which performed a systematic review and grading of evidence related to the screening and treatment of CVD risk factors, including dyslipidaemia, were released in 2011 in a combined effort to improve the assessment and management of cardiovascular disease risk factors [30, 31]. The universal screening for lipid disorders as recommended in the guidelines means that all children between the ages of 9–11 should

Risk factors	Risk conditions
High-level risk factors	*High-risk conditions*
1. Hypertension requiring drug therapy (i.e., BP ≥99th percentile +5 mmHg)	1. Type 1 and 2 diabetes mellitus
2. Current cigarette smoker	2. Chronic kidney disease/end-stage renal disease/ postrenal transplant
3. BMI ≥97th percentile	3. Post-orthotopic heart transplant
4. Presence of high-risk conditions	4. Kawasaki disease with current aneurysms
5. Family history of premature CVD	*Moderate-risk conditions*
Moderate-level risk factors	1. Kawasaki disease with regressed coronary aneurysms
1. Hypertension not requiring drug therapy	2. Chronic inflammatory diseases, such as:
2. BMI ≥95th percentile, but <97th percentile	• Systemic lupus erythematosus
3. HDL-C < 40 mg/dl	• Juvenile rheumatoid arthritis
4. Presence of moderate-risk conditions	3. HIV
	4. Nephrotic syndrome

Adapted from Kwiterovich, P. O., Jr. (2008). Recognition and management of dyslipidaemia in children and adolescents. J Clin Endocrinol Metab, 93(11), 4200–4209 [28].

Table 3.
Risk factors and conditions for dyslipidaemia screening.

get their lipids checked one time. This can be done by determining the plasma level of non-HDL-C with either a fasting lipid profile or a non-fasting test. This universal screening for dyslipidaemia was suggested because studies showed that 30–60 percent of children and adolescents with extreme cholesterol elevations could be missed by using only a selective screening approach based on family history [31].

The universal screening technique is specifically intended to identify children with inherited dyslipidaemia as hypercholesterolemia runs in families. However, due to lifestyle and obesity factors, children with dyslipidaemia, high triglyceride levels and low HDL-C levels may also be identified. In most cases, dyslipidaemias are clinically silent and selective screening for children with family history does not identify a significant number of children with lipid disorders [31, 32].

6. Approach to diagnosis of dyslipidaemia in children

The risk factors for dyslipidaemia are basically those that have been established to increase the likelihood of adults to develop ASCVD as listed in **Table 3**, while risk conditions are specific to paediatric guidelines and involve diseases that increase the risk of developing premature CVD [3]. There will be no noticeable clinical signs and symptoms for most children and adolescents with dyslipidaemia. The lack of clinical features is because, apart from individuals with Homozygous Familial Hypercholesterolemia (HoFH) that may have the first clinical clues in the first 10 years of life, most symptoms and signs only grow after decades [21]. Patients with HoFH are born with elevated LDL-C levels in their blood, and this is one of the reasons behind the early development of serious disease complications [21].

Physical signs, such as lipid deposits, bleeding, and atrophy, occur in the eyes, skin, and tendons. These signs may differ for each person and are therefore not necessarily invariable for the same disease. The heterozygous phenotype is generally present with tendon xanthomas, while the homozygous phenotype is present with both tendon and skin xanthomas. The characteristic lesion of familial hypercholesterolemia is the thickening of the tendons of Achilles. Symptoms caused by these deposits in the tendons and joints include chronic inflammation and joint pain, which can make it difficult for a person to live a good life. Cutaneous xanthelasmas of the eyelids can often be seen in patients with FH, but this symptom is not quite common. It should be noted that the diagnosis of xanthomas in the clinical examination is not only of diagnostic importance but may also signify the possibility of a cardiovascular event, as patients whose xanthomas have been observed in the clinical examination have been shown to suffer from more cardiovascular events. If there is a "white crescentic line" on the skin due to cholesterol accumulation, this also supports the diagnosis of hypercholesterolemia.

Of late, there are arguments surrounding universal screening of children and adolescents for dyslipidaemia, some favour universal screening, while others are against universal screening. While current data still classify approximately one third of children as having elevated lipid levels, some authors have documented the diagnosis of elevated lipid levels in many children without a family history of CVD or hypercholesterolemia [33]. Although it is more common in adults and youth with genetic disorders, selective screening may also be missed in many adults, particularly when their parents are young, free of CVD and unaware of their own lipid levels. Universal screening for these carriers can also be conducted to classify those with undiagnosed heterozygotes for familial hypercholesterolemia or those with more pronounced homozygotes who will then undergo more extensive care, including the possibility of drug therapy. In a meta-analysis, 88–96 percent of all

cases with a false-positive rate of less than 1% were detected in screening for family hypercholesterolemia in the primary treatment clinic [34].

Current literature tells us that risk factors for CVD are most observed in adolescence, and these risk factors are still present in adulthood. Dietary and hygienic treatment and medication are effective for those who have this disease. It is therefore necessary for all children and adolescents to perform a separate lipoprotein test for each of them. However, it can be argued that screening for people who have never smoked should be universal, given the prevalence of obesity in young Africans, the epidemic of metabolic syndrome, and the fact that CVD is quickly becoming a cause of death for individuals under 55 years of age, and will likely be the leading cause of death for all adults under 65 [33].

However, as noted in another publication, concerns have been raised that several longitudinal studies [33] find that when the 75th percentile for triglyceride levels in children is used as a screening cut-off point, only half of those in need of adult treatment are identified by universal lipid screening. In one study, the sensitivity was much lower when screening occurred during puberty, which is likely to indicate a transient downward shift in LDL-C during this time of rapid growth and development [35]. Another unresolved question is whether the detection of elevated TC or LDL-C in children and young adults suggests that these adults will develop premature CVD. If systematic screening for lipid and non-lipid risk factors for CVD was a standard of paediatric treatment, there would obviously be a need for national resources to recognise and treat those found to be at high risk of CVD.

7. Conclusion

Children and adolescents who are predisposed to dyslipidaemia are more likely to remain predisposed to dyslipidaemia throughout their lives. Interventions in childhood and adolescence are immensely helpful in helping to prevent the build-up of fatty deposits in the arteries and other cardiovascular problems later in life. Therefore, abnormalities in the lipid profile of children and adolescents, particularly those with other risk factors, must be identified early, followed, monitored, and treated, if possible. Health care providers should strive to examine, diagnose, and treat prevalent genetic conditions such as familial hypercholesterolemia that affect families for several generations. Lipid screening should be one of the routine therapies for children in Africa.

Author details

Bose Etaniamhe Orimadegun
Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria

*Address all correspondence to: orimadegunbose@yahoo.co.uk

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Chapter 9

Statins for Children with Familial Hypercholesterolemia

Dinara Sadykova, Liliia Galimova, Evgeniia Slastnikova, Zulfiia Khabibrakhmanova and Natalya Guseva

Abstract

Familial hypercholesterolemia (FH) is the most common genetic disorder in the world. It is characterized by increased level of total cholesterol (TC), low-density lipoproteins (LDL-C) since childhood. The diagnosis and initiation of therapy are optimal in childhood before complications (aortic stenosis, atherosclerotic changes in the arterial walls) appear. The initiation of lipid-lowering therapy in FH since childhood is important to reduce the cumulative effect of LDL-C, to increase patient's life expectancy. Statins are recommended as first-line drugs for treatment with monitoring of the recommended clinical, biochemical markers under the supervision of a physician. However, due to limited experience, there are differing opinions among clinicians regarding the age of initiation of lipid-lowering therapy. This review is an attempt to critically study the available data from the world literature concerning the use of statins in children with FH, their effectiveness, safety. It is important to determine the endpoints for determining the effectiveness of statins, such as lowering LDL-C, assessing the thickness of the intima-media complex. The frequency of occurrence of possible side effects in children is considered - diabetes mellitus, hepatotoxicity, muscle pain and others. There is a need to continue randomized trials to prove the lifelong benefit of low LDL-C in patients with FH.

Keywords: children, familial hypercholesterolemia, total cholesterol, low density lipoprotein efficacy, treatment, statins, side effects

1. Introduction

Hypercholesterolemia occupies an important place among the factors of cardiovascular mortality [1]. It is known that the level of lipids in 40–60% of cases is due to genetic reasons [2]. Familial hypercholesterolemia is one of the most common hereditary diseases: its prevalence is 1: 200–1: 500 in the general population [3]. The estimated number of people with a heterozygous form of the disease in Russia should be about 1 million [4]. Despite the high urgency of early detection of the disease, in our country it is diagnosed in less than 1% of the expected number of patients. Diagnosis and initiation of therapy for the disease in childhood are considered optimal before complications such as aortic stenosis, atherosclerotic changes in the arterial walls appear. Statins are recommended for treatment as first-line drugs, but experience with their use in children is limited and requires special analysis.

Statins are 3-hydroxy-3-methyl coenzyme A reductase inhibitors that limit the rate of endogenous cholesterol synthesis. This leads to a decrease in the content of

intracellular cholesterol and the level of circulating LDL-C in the blood. In addition to the lipid-lowering effect, statins affect atherosclerotic plaque (reduce its size, stabilize the surface, thereby reducing the risk of rupture and ulceration), as well as inflammatory factors and endothelial function (pleiotropic effects) [5]. The main goal of prescribing statins in familial hypercholesterolemia is to reduce the risk and rate of development of atherosclerosis and coronary heart disease in order to delay the onset of cardiovascular accidents as much as possible. It should be noted that the use of statins among the adult population, as a rule, is not in doubt, while the pharmacotherapy of hypercholesterolemia in pediatrics raises questions from doctors and parents regarding its effectiveness, long-term prospects and possible complications.

2. Use of statins in world wild practice

The effectiveness of statins in familial hypercholesterolemia can be judged primarily by the degree of decrease in serum LDL cholesterol levels. The European and International Atherosclerosis Societies stated that, based on the available data, the hypothesis of atherogenesis associated with high LDL-C levels is no longer a hypothesis and can be considered a proven fact [6]. Randomized studies show that the effect of LDL-C on the development of atherosclerotic vascular disease is determined not only by the absolute level of LDL cholesterol, but also by its cumulative effect on the arterial wall [6–8]. It is known that the cumulative effect of LDL-C increases with age and is 160 mmol in a healthy person by the age of 55. In the absence of treatment, patients with familial hypercholesterolemia reach this value from the age of 35. It has been shown that starting statin therapy from 18 years of age allows this cumulative load to be postponed to 48 years of age. When statins are taken from the age of 10, the accumulation of LDL-C of 160 mmol is achieved only by the age of 53, which is close to the indicators of healthy people [9].

Demonstrated clinically significant reduction in LDL cholesterol levels in children with familial hypercholesterolemia taking statins compared with children receiving placebo. Moreover, the degree of reduction varied depending on the dose and the drug used. In the work of S.B. Clauss et al. (2005) [10] described the experience of using lovastatin in girls with familial hypercholesterolemia at a dose of 20–40 mg/day for 24 weeks. The authors concluded that in the lovastatin group there was a significant decrease in LDL cholesterol from baseline by 23–27%, total cholesterol by 17–22%, and apolipoprotein B by 20–23%. In another study, pravastatin versus placebo in children with familial hypercholesterolemia younger than 14 years old at a dose of 20 mg/day and over 14 years old - at a dose of 40 mg/day with a duration of therapy of 104 weeks, the decrease in LDL cholesterol levels reached 24.1% [11]. A number of works have been devoted to the experience of using atorvastatin in childhood. It was shown that the appointment of atorvastatin at a dose of 20–40 mg/day compared with placebo for 6–48 months led to a significant decrease in LDL cholesterol by an average of 32–39%, total cholesterol by 32%, triglycerides by 12% and apolipoprotein B by 34% [12–14]. It should be noted that in one of the studies, a statistically significant increase in the level of HDL cholesterol by 2.8% was stated [12]. In a study by H.J. Avis et al. (2010) [15], who studied the efficacy of rosuvastatin in children with familial hypercholesterolemia compared with placebo, showed a decrease in LDL cholesterol, total cholesterol and apolipoprotein B levels for all three doses (5 mg, 10 mg and 20 mg) with a duration of 12 week Thus, in general, the results of studies evaluating the effects of statins in familial hypercholesterolemia demonstrate the effectiveness of statins in lowering LDL cholesterol and total cholesterol levels in children.

3. Effect of statins on the thickness of the intima-media complex

Another important endpoint for determining the effectiveness of statins is the thickness of the intima-media complex, a clinically significant marker of cardiovascular disease. It is noted that the thickness of the intima-media complex in children depends on age, gender and LDL cholesterol level [16]. In children with familial hypercholesterolemia, a much faster increase in this parameter with age was found than in healthy brothers and sisters [17]. Currently, there is a number of studies demonstrating the effect of statins on reducing the thickness of the intima-media complex. M.J. Braamskamp et al. [18] found that in the case of initiation of statin therapy in familial hypercholesterolemia from the age of 12 years, the thickening of the intima-media complex in children occurs more slowly than in peers with familial hypercholesterolemia who do not take statins. The authors concluded that early initiation of statin treatment can delay atherosclerotic changes in the vessels in adolescents and young adults [18]. Statin therapy also has a positive effect on markers of atherosclerosis such as flow-dependent vasodilation. In a study by S. De Jongh et al. (2002) [19], it was found that against the background of 28-week treatment with simvastatin, the flow-dependent vasodilation significantly improved by an average of 4% in children with heterozygous familial hypercholesterolemia compared with that in healthy peers who received placebo.

4. Long-term studies of the effectiveness of statins in childhood

The need for long-term studies to assess the effectiveness of statins in childhood has been repeatedly emphasized. In October 2019, a group of scientists published the results of the longest to date follow-up of children with familial hypercholesterolemia taking statins [8]. 214 patients with this disease and their 95 healthy brothers and sisters were under observation for 20 years. It was found that patients taking statins did not significantly differ from their healthy siblings in terms of an increase in the thickness of the intima-media complex. At the same time, the incidence of cardiovascular diseases and mortality from them at the age of 39 years among patients with familial hypercholesterolemia was lower than among their parents suffering from this disease, and amounted to 1% versus 26% and 0 versus 7%, respectively. These studies make it possible to substantiate the need for the use of statins for the primary prevention of complications and increase life expectancy in children with familial hypercholesterolemia. The effectiveness of treatment depends on the dose of drugs and the age of initiation of therapy. The authors of these and a number of other clinical studies emphasize the need to start statin therapy at the age of 8–10 years [9, 20–23].

5. Features of statins usage in children with FH in clinical practice

In clinical practice, along with an increase in the frequency of statin use, their side effects are increasingly the subject of research. In adults, statin-related side effects include elevated liver transaminases, creatine kinase, and rhabdomyolysis [24]. The main concerns in the pediatric population are the risk of developing myalgias, as well as the possible effect of statins on liver function [13, 19], cholesterol-dependent production of steroid hormones in the gonads and adrenal glands [24] and energy metabolism [25], as well as the child's growth [26].

In a number of studies, when using statins in children with familial hypercholesterolemia, it is noted the occurrence of muscle pain: when taking pitavastatin, they

developed within 3 months in 9.3% cases [18], and when taking pravastatin and atorvasttin - for 48 months in 12.2% of cases [12]. This prevalence of side effects in the form of muscle damage is higher than in adults, who experience myalgia in only 1.5–5% of cases [27]. In this regard, the authors concluded that most of the presented cases of side effects of statins on skeletal muscles in children may not be myalgias directly related to taking statins, but "growing pains" and subjective feelings of the child. A meta-analysis of 6 studies evaluating the efficacy and safety of statins in children with familial hypercholesterolemia with a total of 798 study participants with statin treatment durations from 12 to 104 weeks did not reveal a statistically significant increase in the number of side effects, including myalgias, when prescribing statins compared with placebo [28]. The European Society of Atherosclerosis recommends measuring the level of creatine phosphokinase (CPK) before treatment and 1–3 months after the start of statin therapy [29] in order to control the possible occurrence of myalgias.

The studied side effects, in respect of which remain alert, is hepatotoxicity. Several studies with a total of 943 children with familial hypercholesterolemia taking statins have examined the effect of statins on liver function [30, 31]. The authors emphasize the absence of significant differences in the incidence of violations of the activity of hepatic transaminases in treatment with statins and taking placebo. This confirms the good tolerability of the drugs. The European Society of Atherosclerosis recommends measuring the levels of alanine and aspartate aminotransferases (ALT and AST) before starting statin therapy, and then every 3 months during treatment if there is a history of liver disease or an increase in the level of hepatic transaminases by more than 3 times from the upper limit of normal [32].

Other discussed potential side effects of statins include puberty disorders. Randomized studies evaluating the efficacy and safety of statins in children with familial hypercholesterolemia found no signs of impaired puberty when pravastatin was used at a dose of 20–40 mg/day for 104 weeks [9] and pitavastatin at a dose of 1 mg/2 mg/4 mg/days for 12 weeks [18]. S.B. Clauss (2005) [10] in his article expressed theoretical concerns regarding the use of statins in adolescent girls with potential effects on pituitary hormones (luteinizing hormone and follicle-stimulating hormone), menstrual cycle and physical development. However, in a 24-week study in which adolescent girls with familial hypercholesterolemia took lovastatin 20–40 mg/day, these side effects were not reported [33]. A number of researchers have also demonstrated the safety of using drugs (pravastatin, atorvastatin, rosuvastatin) in children over a similar 2-year period [10, 19, 34].

In recent years, the likelihood of an increase in the risk of developing type 2 diabetes mellitus with prolonged use of statins in adults in the general population has been actively discussed. In a study by J. Besseling et al. (2015), including more than 63 thousand patients with familial hypercholesterolemia taking statins, showed that the prevalence of type 2 diabetes in this group was significantly lower than that of their relatives (1.75% versus 2.93%; $p < 0.05$) [35]. A 10-year prospective follow-up of 194 children with familial hypercholesterolemia receiving statins revealed one new case of type 2 diabetes mellitus without significant differences in morbidity in their 83 siblings without familial hypercholesterolemia [36]. N. Joyce et al. (2017) [37] also showed no significant differences in the incidence of type 2 diabetes mellitus among children taking statins compared with children not receiving drugs in this group.

Thus, it should be noted that the occurrence of side effects when using statins in children cannot be completely ruled out. However, the analysis of studies carried out in this direction emphasizes the low probability of their occurrence. The key when prescribing statins to a child with familial hypercholesterolemia is careful

monitoring of complaints, clinical condition, and a number of biochemical markers in the blood (ALT, AST, CPK). It is also necessary to monitor the patient's condition with prompt correction of the drug and the dose received by the child.

Separate sections devoted to the use of statins in childhood are presented in the most frequently cited clinical guidelines for the diagnosis and treatment of dyslipidemia: American [38], Japanese [20] and Australian [21]. In 2019, the results of a study conducted in 8 European countries (Norway, Great Britain, Czech Republic, Portugal, Greece, Austria, the Netherlands, Belgium) were published, which compared the tactics and results of treatment of familial hypercholesterolemia in a total sample of 3064 children. It has been shown that the proportion of children taking statins increases with age and by the age of 15, already 79% of patients in these countries are taking statins [39]. The goals for children over 10 years of age are to achieve LDL cholesterol <3.5 mmol/L (<135 mg/dL), at a younger age - to reduce this indicator by ≥50%. In the United States and Europe, simvastatin, lovastatin, atorvastatin, pravastatin, fluvastatin, and rosuvastatin are approved for use in children with familial hypercholesterolemia. In the United States, all of these statins are approved from the age of 10, with the exception of pravastatin, which is recommended from the age of 8. In Europe, rosuvastatin is approved from 6 years old, in Australia atorvastatin is approved in children from 6 years old. It is recommended to start statin therapy with low doses and increase it until the set goals are achieved [29] with a possible increase to the maximum admissible dose in childhood established for each of the drugs. This dose is 20 mg for pravastatin for children under 13 years of age and 40 mg for children under 18; for rosuvastatin - 10 mg up to 9 years and 20 mg - up to 18 years; for atorvastatin - 40 mg regardless of age [12].

The results of recent randomized studies on the efficacy and safety of statins in children with familial hypercholesterolemia formed the basis for the joint recommendations of the European Society of Cardiology and the European Society of Atherosclerosis [29]. In 2018, Russian guidelines for the diagnosis and treatment of familial hypercholesterolemia were published [40]. These recommendations serve as the main document in the work of a pediatric cardiologist and pediatrician when monitoring children with familial hypercholesterolemia. According to these recommendations, statin therapy should be considered in children aged 8–10 years with heterozygous disease.

Particular vigilance should be shown in relation to children with a homozygous form of familial hypercholesterolemia, in which lipid-lowering therapy should be started as early as possible, immediately after the diagnosis. It is recommended to prescribe therapy with maximum tolerated doses of statins in combination with other lipid-lowering drugs in order to maximize the reduction of LDL cholesterol [40].

6. Conclusions

Thus, the initiation of lipid-lowering therapy in familial hypercholesterolemia from childhood is of great importance for reducing the cumulative effect of LDL cholesterol and increasing the patient's life expectancy. When answering questions regarding the treatment of children with dyslipidemia, one should take into account the compelling reasons to follow international recommendations and use statins for familial hypercholesterolemia from the age of 8–10 years, with monitoring of the recommended clinical and biochemical markers under the supervision of a physician. There is currently a need to continue randomized trials to prove the lifelong benefit of low LDL cholesterol in patients with familial hypercholesterolemia.

Acknowledgements

We want to thank the Head of Tatarstan Republic's Ministry of Health, the Rector of Kazan State medical University, Kazan, Russian Federation, the Head of the Children's Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan, Russian Federation and for the help in the organization of Children's Lipid Center, where we run our observation of children with Familial Hypercholesterolemia.

Conflict of interest

The authors declare no conflict of interest.

Author details

Dinara Sadykova[1,2*], Liliia Galimova[1,2], Evgeniia Slastnikova[1,2], Zulfiia Khabibrakhmanova[1] and Natalya Guseva[1,2]

1 Kazan State Medical University, Kazan, Russian Federation

2 Children's Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan, Russian Federation

*Address all correspondence to: sadykovadi@mail.ru

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Section 3

Treatment of Dyslipidemia

Chapter 10

Changes in Atherosclerotic Plaque Composition with Anti-Lipid Therapy as Detected by Coronary Computed Tomography Angiography

Drew Thomas, Darma Marcelin and Shone Almeida

Abstract

Lipid management remains the mainstay of cardiovascular disease prevention. Drugs that target cholesterol reduction, such as HMG-CoA reductase inhibitors (statins) and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, have shown significant mortality and morbidity benefit. Predominantly targeting low-density lipoprotein (LDL). These drugs have been indicated to reduce lipid composition and plaque proliferation. Total plaque burden and composition can now be assessed with noninvasive advanced cardiac imaging modalities. This chapter will address the components of atherosclerotic plaque as identified with coronary computed tomography angiography (CCTA) and review in detail the changes in plaque characteristics that may be responsible for reduction in cardiac events. These changes in plaque composition may help guide future management of cardiovascular disease, serving as an imaging biomarker for better risk stratification. Readers will gain a deeper understanding of plaque morphology with direct clinical applicability as well as an understanding of how noninvasive imaging can be utilized to assess plaque composition.

Keywords: coronary computed tomography angiography (CCTA), statin, PCSK9, low density lipoprotein (LDL), atherosclerotic plaque

1. Introduction

Cardiovascular disease (CVD) has remained the leading cause of death in the United States in 2018 [1]. Atherosclerosis is the leading factor in causing cardiovascular death. The main factors that drive atherosclerosis are LDL cholesterol, hypertension, diabetes, and smoking [2]. Currently the United Stated Preventive Services Task Force recommends screening everyone who are at risk for coronary artery disease, all men over age 35 and all women over age 45. For risk stratification a ten year atherosclerotic risk score can be calculated to help decide if treatment should be started [3]. Treatment options can be aided by cardiac imaging. Cardiac imaging is mainly performed via intravascular ultrasound (IVUS), optical coherence tomography (OCT), and coronary computed tomography angiography (CCTA).

This development has made plaque characterization possible. Categories of plaque are placed into 4 broad categories: low-attenuating, fibrofatty, fibrocalcified, and densely calcified plaque [4].

2. Pathophysiology of atherosclerosis

The coronary arteries are composed of three layers: tunica intima, tunica media, and tunica adventitia. The tunica intima is the innermost lining inside these vessels, which is further composed of three layers of the endothelium. Simple squamous cells make up the innermost layer, which functions as a barrier that allows blood to flow smoothly. Any break in any part of the endothelial lining exposes blood to the subendothelial layer, starting the clotting mechanism. The tunica media is the middle layer of the vessels, composed of layers of smooth muscle cells. The tunica adventitia is the outer connective tissue layer supporting the vessel itself formed of loose connective tissue, vessels, and nerves [1]. When the initial fatty streaks or plaque precursors grow, it is usually outward, meaning there is not an initial direct effect on the blood flow or size of the lumen [5]. Lesions progress when the intima's smooth muscle cells begin to divide and secrete extracellular matrix molecules, such as collagen [6]. Other smooth muscle cells will penetrate the intima from the media, where they will join the other muscle cells in forming a fibrous cap overlying the lipid core. When there is a persistence of risk factors such as high LDL levels, the lipid core continues to grow in this manner.

The major lipoprotein classes include chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins, low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Larger in size and more triglyceride-rich lipoproteins are the less dense. Protein molecules, known as apolipoproteins (apo) on these particles' surface, guide the lipoproteins' interaction with tissues, cells, and different organs. LDL receptors bind to apo-B, the predominant apoprotein in LDL, and apo-E found on VLDL and some HDL particles. This receptor-mediated uptake delivers circulating cholesterol to the liver and other tissues. Apo-B may contribute to LDL's atherogenicity by promoting Apo-B particle entrapment within the arterial wall. Apo A-I, the predominant apoprotein in HDL, aids antiatherogenic efflux of cholesterol from peripheral tissues [6].

The different type of plaque morphology includes foam cell-rich matrix (obtained from fatty streaks), collagen-rich matrix (from sclerotic plaques), collagen-poor matrix without cholesterol crystals (from fibrolipid plaques), atheromatous core with abundant cholesterol crystals (from atheromatous plaques), and segments of normal intima derived from human aortas at necropsy. Atheromatous cores are associated with the most significant platelet deposition and largest thrombus formation than other components of atherosclerotic lesions. Therefore, we are led to believe that atherosclerotic plaques with larger atheromatous cores are more prone to cause acute coronary events because of their greater thrombogenicity after rupture [7].

Studies have also shown that aggressively lowering LDL cholesterol alters the composition of coronary atheromas. Lowering LDL cholesterol decreases the size of the lipid core, stabilizing the plaque by decreasing the lipid core and increasing the ratio of fibrous tissue to atheroma. This stabilizes the plaque by increasing the calcification of the plaque, reducing likelihood of plaque rupture [6]. There is an increase in fibrous tissue and calcified tissue within the atheroma replacing the lipid core. Therefore, calcification increases with aggressive lipid-lowering therapies like statins.

Noninvasive imaging modalities such optical coherence tomography (OCT), intravascular ultrasound (IVUS), and coronary computed tomography angiography (CCTA) have the ability to characterize plaque based on the above pathophysiology and define high risk features that in turn increase risk of cardiovascular events [8].

3. Plaque characterization by noninvasive imaging

Coronary artery calcium scoring and coronary artery calcification assessment are among the most emerging noninvasive coronary artery imaging applications. Initially, calcifications on chest radiography and ex vivo histology were used to discuss the relationship between coronary calcification and obstructive coronary artery diseases. The coronary artery calcium (CAC) scan consists of a non-contrast, electrocardiogram gated computed tomography of the heart. It is obtained during a short period of held inspiration. Once the image is obtained, the arterial calcium is defined using Hounsfield units. A density of greater than 130 Hounsfield units across an area of at least 1 mm is considered significant. Atherosclerotic calcification is reported either by volume or mass in Agatston units (AU), a semi-quantitative measure that further incorporates aspects of calcium density and distribution [9].

Progression of coronary calcium scores have a direct impact on clinical coronary events. One of the largest cohorts, the Multi-Ethnic Study of Atherosclerosis (MESA), followed over 5,600 patients. Participants received initial CAC scans and were followed for a median duration of 7.6 years, and then received follow up CAC. The change in calcium score was measured between initial and follow up was correlated with coronary artery events. Endpoints included myocardial infarction, angina followed by revascularization, resuscitated cardiac arrest, and cardiac death. Patients with non-zero CAC score at baseline were more likely to be older, male, diabetic, previous smoker and be on lipid lowering medication or hypertensive medication. Eighty-four percent of patients with a zero CAC score at baseline remained at zero on follow up. Patients with calcium score increases greater than 100 had a two to three-fold greater risk for the cardiac endpoints. Patients with 15–29% annual increase in CAC had an increased risk (hazard ratio: 1.6) for cardiac events compared to those with less than five percent progression annually. The study concluded that CAC scores correlate with clinical events including MI and cardiac death [10].

Computed tomography can characterize plaque morphology based upon Hounsfield Units, identifying high risk plaque. Plaque morphology on CCTA is characterized as low-attenuating plaque, fibrofatty, fibrocalcified and densely calcified. High risk or vulnerable plaque features include low-attenuating, spotty calcification and positive remodeling. Compared with intravascular ultrasound,, CCTA has shown high sensitivity and specificity in evaluating plaque morphology [4].

A large study published in the Journal of American College of Cardiology by Motoyama et al. established CCTA characterization of plaque morphology and the clinical implications. The study stratified patients into different morphological groups. Low attenuated plaque was defined as less than 30 Hounsfield Units. This was correlated with previous studies using intravascular ultrasound showing a sensitivity of 91% and specificity of 100%. %. Intermediate attenuated plaque was defined 30 HU to 150 HU and calcified plaque was defined as greater than 150 HU. Remodeling was the other factor in this study (positive, negative, or none). Coronary artery positive remodeling was defined as when the size of the lumen is increased by 10% more in the region of the plaque than in a reference segment

proximal to the plaque. This large study showed that when a subject has both positive remodeling and low attenuated plaque they are at high risk for having an acute coronary syndrome in the next two years. Twenty two percent of subjects with both low attenuated plaque and positive remodeling had acute coronary syndrome within the next two years compared to less than one half of one percent of the subjects with neither positive remodeling nor low attenuated plaque [11].

Several trials have demonstrated that use of statin therapy has shown benefit in primary prevention of coronary artery disease and secondary prevention of coronary artery disease. Statins have become the standard of care in coronary artery disease and are in all major anti lipid and coronary artery disease guidelines. Statins offer several benefits. Directly, they reduce hepatic cholesterol synthesis but several pleiotropic effects have been defined, including reduction in inflammation, cholesterol egress from the vasculature and plaque stabilization as described. There are many different areas of research being performed on various potential beneficial effects of statin therapy including beneficial effect on vascular tone by the upregulation of nitric oxide, reducing platelet aggregation and having antithrombotic properties, and anti-inflammatory properties including reduction in oxidative stress [4].

4. Changes in atherosclerotic plaque composition with lipid lowering therapies

4.1 Statins

Cardiovascular disease (CVD) has remained the leading cause of death in the United States in 2018 [9]. One of the main targets in the biosynthesis of cholesterol is inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA). Reduction in low density lipoprotein (LDL) cholesterol and then therefore reduction in morbidity and mortality has been achieved in several trials including Scandinavian Simvastatin Survival Study and West of Scotland Prevention Study [4].

Computed tomography can characterize plaque morphology based upon Hounsfield Units. This can identify at risk plaque. Plaque morphology on CCTA is identified as low-attenuating, fibrofatty, fibrocalcified, and densely calcified. At risk or vulnerable plaque is low-attenuating plaque. Intravascular ultrasound is the gold standard in characterizing plaque, however CCTA has shown high sensitivity and specificity in evaluating plaque morphology [10].

A large study published in the Journal of American College of Cardiology by Motoyama et al. established CCTA characterization of plaque morphology and the clinical implications. The study stratified patients into different morphological groups. Low attenuated plaque was defined as less than 30 Hounsfield Units. This was correlated with previous studies using intravascular ultrasound showing a sensitivity of 91% and specificity of 100%. %. Intermediate attenuated plaque was defined 30 HU to 150 HU and calcified plaque was defined as greater than 150 HU. Remodeling was the other factor in this study (positive, negative, or none). Coronary artery positive remodeling was defined as when the size of the lumen is increased by 10% more in the region of the plaque than in a reference segment proximal to the plaque. Therefore, a subject could have both low attenuated plaque and positive remodeling, either low attenuated plaque or positive remodeling, or neither low attenuated plaque nor positive remodeling. This large study showed that when a subject has both positive remodeling and low attenuated plaque they are at high risk for having an acute coronary syndrome in the next two years.

Twenty two percent of subjects with both low attenuated plaque and positive remodeling had acute coronary syndrome within the next two years compared to less than one half of one percent of the subjects with neither positive remodeling nor low attenuated plaque Several trials have demonstrated that use of statin therapy, HMG-CoA reductase inhibition, has shown benefit in primary prevention of coronary artery disease and secondary prevention of coronary artery disease. Statins have become the standard of care in coronary artery disease and are in all major anti lipid and coronary artery disease guidelines. They also have mortality benefits as well. Statins work in the pathway of cholesterol synthesis. This occurs in the hepatocytes. This results in upregulation of hepatic LDL receptors and reduces serum LDL. This causes reduction in LDL deposition on atherosclerotic plaque on coronary arteries and other arteries for that matter. Statins also have reduction in the inflammatory cascade as well. Overall there is stabilization of atherosclerotic plaque due to statin therapy. There are several trials including Rosuvastatin to Prevent Vascular Events in Men and Women With Elevated C-Reactive Protein (JUPITER), Effects of Atorvastatin on Early Recurrent Ischemic Events in Acute Coronary Syndromes (MIRACL), and Heart Outcomes Prevention Evaluation (HOPE-3) that all show reduction in C-Reactive Protein (CRP). This also could aid in reduction of coronary events but is still controversial at this point. There are many different areas of research being performed on various potential beneficial effects of statin therapy including beneficial effect on vascular tone by the upregulation of nitric oxide, reducing platelet aggregation and having antithrombotic properties, and anti-inflammatory properties including reduction in oxidative stress [10] Atherosclerotic plaque can regress. Plaque regression occurs when there is removal of lipids and necrotic material from the plaques, endothelial repair occurs, and smooth muscle proliferation stops. This can occur by several different mechanisms: high density lipoprotein cholesterol, destruction of foam cells and macrophages, and restoration of endothelium. Statin therapy has been studied in the process of atherosclerotic plaque reduction. Meta-analysis of eight trials comparing statin with placebo showed that in 919 patients with 461 patients in the statin arm and 458 patients in the placebo group that there was a statistically significant mean difference in coronary atheroma volume between the two groups. -3.573 (95% CI -4.46 to −2.68; $P < 0.01$). Plaque characterization was similar in both groups. Intravascular ultrasound was used in the studies [12].

Coronary CTA has allowed physicians to directly visualize atherosclerotic plaque characterization and volume. This allows identification of features of vulnerable plaque including positive remodeling and low attenuating plaque, altering clinical management [4]. One early study on the effects of statin therapy via coronary CTA by Zeb et al. evaluated one hundred patients who underwent coronary CTA without known history of coronary artery disease. Patients underwent plaque characterization as low attenuation plaque (LAP as less than 30 Hounsfield units), non-calcified plaque (NCP) and calcified plaque and volumetric quantification among a statin versus non-statin arm. This was a retrospective observational study in attempts to evaluate the plaque volume and characteristics over time. At mean follow up of 406 days the total plaque volume was reduced in the statin arm compared to the non-statin arm (−33.3 mm^3 +/− 90.5 vs. 31 mm^3 +/− 84.5, $p = 0.0006$). Non calcified plaque volume was significantly reduced in the statin therapy arm as well (−47.7 mm^3 +/− 71.9 vs. 13.8 mm^3 +/− 76.6, $p < 0.001$). Low attenuated plaque volume was shown to have significantly reduced progression (−12.2 mm^3 +/− 19.2 vs. 5.9 mm^3 +/− 23.1, $p < 0.0001$). There was a non-significant trend toward increased production of calcified plaque volume. Mean plaque volume difference was statistically significant for both low attenuated plaque and non-calcified plaque (−18.1, 95% Cl: −26.4, −9.8 for LAP; −101.7, 95% Cl: −162.1, −41.4 for NCP; $p < 0.001$).

The authors concluded that statin therapy lowers the progression of LAP and NCP plaque in comparison with non-statin therapy [13].

Another landmark trial using CT derived plaque characterization was the Paradigm study (Progression of Atherosclerotic Plaque Determined by Computed Tomographic Angiography. This multinational, observational prospective study evaluated serial coronary CTA's at a greater than two-year interval. With 654 enrolled patients, the majority of patients were on statin therapy (408 vs. 246). All patients had baseline coronary CTA's, followed for greater than two years, and then had subsequent coronary CTA. In the statin arm initial low density lipoprotein (LDL) cholesterol was on average 114 and reduced to 94.5 at follow up. The non-statin arm had an initial average LDL of 111 and follow up LDL of 104. The plaque was characterized into calcified and non-calcified plaque including: fibrous, fibro-fatty, and lipid rich. The authors demonstrated that an increase in coronary artery calcium score was associated with the increase in plaque volume in both the statin and non-statin groups. In the non-statin arm coronary artery calcium score was associated with increase in calcified and non- calcified plaque volume increase [B (95% CI): 1.579 (1.150–2.009) and 0.369 (0.123–0.614), respectively, all $P < 0.05$). In the statin arm there was a statistically significant increase in coronary artery calcium score associated with increased calcified plaque volume [B (95% CI): 0.756 (0.552–0.961), $P < 0.001$]. Non calcified plaque volume decreased [B (95% CI): −0.194 (−0.364 to −0.023), $P = 0.026$). Fibrous plaque volume also decreased as well [B (95% CI): −0.304 (−0.535 to −0.073), $P = 0.010$]. The fibro fatty and lipid rich plaque volume also decreased but was not statistically significant. In the non-statin arm, the increase in coronary calcium score reflects the increased atherosclerotic burden including calcified and non-calcified plaque. The statin arm increase in coronary calcium score only is correlated with calcified plaque. Therefore, in the statin arm, while the calcified plaque increased the fibrous and overall, non-calcified plaque decreased. With statin therapy, the ratio of plaque composition shifts to more stable, lower risk morphology, specifically calcified and fibrocalcified types [14].

Another variable of coronary atherosclerosis is the fibrous cap. A thin fibrous cap can also be a sign of vulnerability, increasing risk for plaque rupture and presenting with acute coronary syndrome [15]. The EASY-FIT study (Effect Of Atorvastatin Therapy On Fibrous Cap Thickness In Coronary Atherosclerotic Plaque As Assessed By Optical Coherence Tomography) assessed the differences between Atorvastatin 5 mg and Atorvastatin 20 mg on the fibrous cap using optical coherence tomography. The EASY-FIT study enrolled 70 patients with unstable angina pectoris who had untreated hyperlipidemia. These patients were previously treated with successful percutaneous coronary intervention (PCI). The OCT that was performed looked at a nonculprit lesion in the coronary artery. Patients were placed in either an Atorvastatin 20 mg/day arm or a 5 mg/day arm with OCT at baseline and at 12-month follow-up. Thin-cap fibroatheroma was defined as plaque having minimal fibrous cap thickness of <65 micrometers and thick cap was defined as fibrous cap thickness > 65 micrometers. The fibrous cap thickness increased significantly in both groups. In the Atorvastatin 20 mg/day vs. 5 mg/day the increase was (69% [IQR: 25% - 104%] vs. 17% [IQR: −1% to 34%] $p < 0.001$. The minimal lumen area did not change at follow up for either group. There was a negative correlation between the fibrous cap thickness and the serum LDL but no correlation between total serum cholesterol, triglycerides, or glycosylated hemoglobin. This study suggests that high to moderate intensity statin might be superior in providing benefit in plaque stabilization compared to low intensity Atorvastatin in secondary prevention in the setting of unstable angina [16].

5. Effects of EPA on atherosclerotic plaque via CCTA

There is limited evidence about the effects of eicosapentaenoic acid (EPA) on atherosclerotic plaque. The mechanism of action of these omega 3 fatty acids occurs several ways: reducing inflammation, plaque volume, membrane stabilization as well as triglyceride reduction. There is some evidence that EPA might have an effect on atherosclerotic plaque volume and characterization. The Evaporate trial (Effect of icosapent ethyl on progression of coronary atherosclerosis) was a multicenter, randomized, double-blinded, placebo-controlled trial with intention to treat analysis which evaluated patients with known coronary artery disease and hypertriglyceridemia already on statin therapy. Patients in the EPA arm were given four grams of EPA daily and their plaque progression was measured by CCTA. They had CCTA scans at baseline, 9 months and 18 months. The primary end point was the change in low attenuated plaque volume seen on CCTA. Low attenuated plaque volume was significantly reduced in the EPA arm of the study at 18 month follow up vs. placebo (-0.3 +/− 1.5 vs. 0.9 +/− 1.7 mm^3, $P = 0.006$). Other parameters EPA arm vs. placebo arm respectively: total plaque (−9% vs. 11% $P = 0.002$), total non-calcified plaque (−19% vs. 9%, $P = 0.0005$), fibrofatty (−34% vs. 32%, $P = 0.0002$), fibrous (−20% vs. 1%, $P = 0.003$), and calcified plaque (−1% vs. 15%, $P = 0.053$). Dense calcium did not show significant difference between the two groups. Interestingly there was no significant difference in total cholesterol, LDL, HDL or triglycerides compared to baseline. The study concluded that EPA was associated with plaque regression, specifically the low attenuation, higher risk plaque, compared to statin therapy alone [17].

Of critical importance is whether the change in plaque composition with addition of EPA translates into a reduction in cardiovascular events. The REDUCE-IT trial (Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial) is a randomized, double blinded, placebo controlled trial comparing EPA (4 g total) to placebo. Over eight thousand patients were enrolled and followed for an average of 5 years. In this trial enrollment only occurred for age 45 and older with cardiovascular disease or were age 50 or older with diabetes and at least one other cardiovascular risk factor. Requirements also included: fasting triglyceride level of 150–499 mg/dL, LDL cholesterol level of 41–100 mg/dL, and on the same statin dose for at least four weeks. The primary end point was composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina in time to event analysis. Secondary end points included: key secondary efficacy end point, composite of cardiovascular death or nonfatal MI, fatal or nonfatal MI, emergency or urgent revascularization, cardiovascular death, hospitalization for unstable angina, fatal or nonfatal stroke, death from any cause, non-fatal MI or non-fatal stroke. The primary end-point occurred in 17.2% of patients in the icosapent ethyl arm compared to 22% in the placebo arm (hazard ratio, 0.75; 95% confidence interval, 0.68 to 0.83; $P < 0.001$), absolute between group difference of 4.8% (95% CI, 3.1 to 6.5). The number needed to treat in order to avoid one primary end point was 21 over a 5 year follow up. Key secondary end points occurred in 11.2% of patients in the icosapent ethyl arm compared to 14.8% in the placebo arm (hazard ratio, 0.74, 95% CI, 0.65 to 0.83; $P < 0.001$) with absolute between group difference of 3.6% (95% CI, 2.1 to 5.0). The number needed to treat to avoid one key secondary end point was 28 (95% CI, 20 to 47) in a 5 year follow up. For individual primary and secondary end points icosapent ethyl arm vs. placebo arm respectably: rate of cardiovascular death (4.3% vs. 5.2%; 0.80; 95% CI, 0.66 to 0.98; $P = 0.03$), death from any cause (6.7% vs. 7.6%, 0.87; 95% CI, 0.74 to 1.02). All primary and secondary end points significantly statistically favored

the icosapent ethyl arm compared to placebo except for death from any cause. The study authors concluded that in patients 45 years and older with hypertriglyceridemia receiving statin therapy, cardiovascular risk including cardiovascular death was lowered with icosapent ethyl therapy than with placebo [18].

6. Effects of PCSK9 inhibitors on atherosclerotic plaque via CCTA

Statin therapy has been the standard for the reduction of LDL cholesterol for fifty plus years. However, there is still cardiovascular risk even after high dose statin therapy. To reduce this risk more medications have been developed and implemented. One class is the Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Studies have shown that PCSK9 inhibitors reduce LDL cholesterol and reduce clinical events. An initial study looking at plaque via intravascular ultrasound called the Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound (GLAGOV) showed that there was decrease in percent atheroma volume after 76 weeks with PCSK9 therapy in addition to statin therapy. The primary end point of percent atheroma volume did not decrease in the placebo group but did decrease in the evolucumab group (0.05% vs. 0.95%). The between group difference is −1.0% (95% CI, −1.8% to −0.64%) $P < 0.001$ [19].

In this next trial coronary calcium score was mainly used in combination with coronary CTA to evaluate plaque production in three groups: statin, statin plus PCSK9 inhibitor, and no therapy. This study was a retrospective study to evaluate if PCSK9 inhibitors could reduce the plaque burden that is already present in patients. This was performed by evaluating the association between PCSK9 inhibitor therapy and coronary calcium score via CCTA. Baseline characteristics of the 120 patients that were retrospectively enrolled in this study showed that 53% of patients had calcium score of zero, 47% of patients had coronary calcium score of greater than zero. Twelve and a half percent had severe coronary artery calcification with scores >400 AU's. A correlation was observed between coronary calcium score and lipid lowering therapy ($P < 0.0001$) without correlation between any other traditional risk factor for coronary artery disease including hemoglobin A1c levels, blood pressure, and lipid levels. Scores increased with age ($P = 0.048$) particularly age greater than sixty years old. Coronary artery calcium score was higher in the statin and statin plus PCSK9 group mainly because they had higher diagnosed coronary artery disease and therefore required lipid lowering therapy. Calcium score was measured annually. The progression in the statin monotherapy group was 29.7% and 14.3% in the PCSK9 inhibitor group ($P < 0.01$). There is a clear association between the lower plaque production via coronary calcium score due to PCSK9 therapy. This is a low powered study with a low patient enrollment. There were several limitations including being an open label design, short study duration, all participants were Japanese, and difference in statin dose. More studies need to be performed to include a longer term, double blinded, prospective study. However, in this initial study coronary artery calcium score was reduced from 29.7% in the statin group and 14.3% in the statin plus PCSK9 group. Plaque progression might be prevented by additional therapy with PCSK9 inhibitor therapy [20]. This study can set the stage for future studies using coronary CTA to follow the atherosclerotic plaque with PCSK9 inhibitor therapy. Characteristics of atherosclerotic plaque should also be reviewed in future studies.

Patients with certain comorbidities in addition to coronary artery disease are at higher risk for further acute coronary syndrome events. One comorbidity being diabetes mellitus. The American Diabetes Association is also interested in reducing cardiovascular risk for their subset of patients. In the 457-P: One-Year

Administration of Anti-PCSK9 Antibody Is Enough to Stabilize Vulnerable Coronary Plaques in Diabetic Patients, which Are Resistant to Intensive Statin Therapy trial they attempted to review the efficacy of PCSK9 therapy. As previously described low attenuating plaque is categorized as vulnerable plaque and is associated with increased risk of acute coronary syndrome. In this study 142 patients with type two diabetes mellitus, asymptomatic coronary artery disease with vulnerable coronary plaques, on intensive statin therapy for two years were evaluated by coronary CTA to evaluate the plaque. PCSK9 inhibitors were given for a one-year duration. There was a 74% decrease in LDL cholesterol. Importantly an improvement of vulnerable plaque was seen. This was measured by Housfield Units (HU). As explained previously vulnerable plaque or low attenuating plaque have a Housfield Unit of less than 50. There was a rise in plaque HU (43.2 +/− 12.0 HU at baseline to 128.5 +/− 52.3 HU, $p < 0.0001$ after one year of PCSK9 therapy). This infers stabilization of vulnerable plaque in the setting of diabetic patients on intensive statin therapy with vulnerable plaque seen on CCTA. The conclusion was drawn that a one- year administration of PCSK9 inhibitor therapy produced significant stabilization of vulnerable plaque in patients with asymptomatic coronary artery disease who are resistant to intensive statin therapy [21].

Overall there is very minimal data in this category. This is an area for future studies. Many studies are currently being performed in this area and will continue to explore this area of research. Plaque progression, plaque volume, plaque characterization can all be assessed via coronary CTA. This gives cardiologists advantages in treatment options and more informed discussion with patients. Further research in this area is needed.

7. Future directions

There are several areas for future research. One is epicardial fat and pericoronary fat. For example, in the trial Epicardial adipose tissue and pericoronary fat thickness measured with 64-mutidetector computed tomography: potential predictors of the severity of coronary artery disease they looked at epicardial and pericoronary fat to see if there was association between the amount of fat thickness to the severity of coronary artery disease. This showed that there was in increase in fat thickness in patients with obstructive coronary artery disease [22]. This research is in the beginning stages and much further research can be done.

A new software has been developed called Cleerly software that uses artificial intelligence to quantify coronary artery stenosis and plaque composition at the vessel and lesion level in order to track it over time. This is another clinical tool that is taking an invasive procedure like a coronary artery catheterization and making it noninvasive and easily accessible to patient and physician on an online platform. This helps clinicians determine the best treatment approach for each individualized patient. This is a very small part of a bigger overarching vision of precision medicine.

8. Conclusion

Atherosclerosis is an evolving field with an explosion of data coming recently of various subsets which include different imaging modalities, plaque characterization, and treatment options. This has allowed the medical field to delve into the pathophysiology of atherosclerotic plaque formation, how to properly image this plaque, and proper ways to treat this plaque in order to lower clinical events and

mortality. The coronary CTA has allowed us to look at the morphology of atherosclerotic plaque and to be able to track the plaques over time on various treatments including statin therapy which is the gold standard, icosapent ethyl therapy, and the newest treatment of PCSK9 inhibitors. Many trials as discussed above have shown the effects of various medications on atherosclerotic plaque over time and how it correlates to the morphology of the plaque and how this correlates clinically. The morphology change from a high risk plaque to a low risk plaque with a higher calcification is one of the key discoveries that coronary CTA allowed us to see. The non- invasive approach of this modality is clearly the future of following atherosclerosis in patients on lipid lowering therapy. Future research needs to be performed on the effects PCSK9 inhibitors on atherosclerotic plaque via coronary CTA as well as continued trials connecting clinical events and mortality to lipid lowering therapy via coronary CTA.

Author details

Drew Thomas[1*], Darma Marcelin[1] and Shone Almeida[2]

1 HCA Midwest Internal Medicine GME, Overland Park, Kansas, USA

2 HCA Midwest Heart and Vascular Specialists, Overland Park, Kansas, USA

*Address all correspondence to: drew.thomas@hcamidest.com

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Chapter 11

Effect of Tomato Juice Supplements Consumption on the Lipid Profile of Dyslipidemia Patients

Sugini

Abstract

The objective of this study was to determine the effect of tomato juice supplements consumption on the lipid profile of women with dyslipidemia patients. The design of this study was a quasi-control experimental design with pre-post test. The subjects were sixty-two Kendal Hospital women employees, aged 35–50 years old, suffering from dyslipidemia but did not suffer from metabolic or degenerative diseases based on the examination of a specialist in internal medicine. Subjects were divided into two groups, group I (30 people) were given antioxidant supplements (336 g of tomatoes per day) for 21 days and group II (32 people) as control. The nutrition intake data was measured by the 24-hour food withdrawal method conducted for three consecutive days in three weeks of research calculated by the Nutrisurvey program. Data was analyzed with Kolmogorov Smirnov test, Pearson test and Mann Whitney test. There were significant relationships between energy intake, protein intake, fat intake, and carbohydrate intake with total cholesterol levels and triglyceride levels and there were also significant relationships between energy intake and fat intake with low density lipoprotein levels. It shows that there is a relationship between diet and dyslipidemia. There were significant differences in total cholesterol, low lipoprotein levels and triglycerides in treatment and control groups. This showed that tomato juice supplements significantly affect the lipid profiles.

Keywords: diet, tomato juice supplements, lipid profile, cardiovascular disease

1. Introduction

The relationship between blood cholesterol and heart disease has been well-established, with a decrease in Cholesterol Low Density Lipoprotein (LDL) being the main target of preventive therapy. High levels of LDL cholesterol in blood vessels cause these molecules to penetrate the sub-endothelial space which can be easily oxidized by free radicals. This oxidized LDL causes damage to nearby structures, causing the recruitment of monocytes to eliminate the oxidized LDL. This elimination process produces by-products in the form of foam cells. These foam cells release toxins that cause damage to endothelium cells, hypertrophy, and smooth muscle hyperplasia of blood vessels. This process also stimulates platelet

aggregation which can interfere with the production or availability of nitric oxide which results in decreased blood vessel lumen and ischemia in tissues and organs. As this inflammatory process develops, high LDL cholesterol levels can develop into atherosclerosis [1].

Cardiovascular disease (CVD) is a leading cause of human disability and premature death worldwide. Diet has a direct relationship with the development of CVD and dietary changes are the current approach CVD prevention which is to increase consumption of fruits and vegetables as a good source of some antioxidant phytochemicals, e.g. carotenoids. Lycopene is the most abundant carotenoid in tomatoes and tomato products have gained profuse attention in recent years for their beneficial of health role, especially those related to their antioxidant effects and its protective role against CVD [2]. Dietary and antioxidant supplements are efforts to prevent the development of dyslipidemia into cardiovascular disease. The 2015 US Diet Guidelines Advisory Committee summarizes the evidence for the benefits of a healthy diet on cardiometabolic outcomes and other diseases and also describes a healthy vegetarian diet, which includes more peas, soy products, nuts and seeds, and seeds. Whole grains but does not include meat, poultry, or seafood [3]. Women in the top aMed quintile have a lower risk for both Coronary Heart Disease (CHD) and stroke than bottom quintile (RR = 0.71 (95% CI = 0.62–0.82; p trend<0.0001) for CHD; RR = 0.87 (95% CI = 0.73–1.02; p trend = 0.03) for stroke). CVD mortality was significantly lower among women in the top quintile of the aMed (RR = 0.61, 95% CI = 0.49–0.76, p trend<0.0001) [4]. Lycopene may improve vascular function and contributes to the primary and secondary prevention of cardiovascular disorders. The main activity profile of lycopene includes anti-atherosclerotic, antioxidant, anti-inflammatory, antihypertensive, antiplatelet, antiapoptotic, and protective endothelial effects, the ability to improve the metabolic profile, and reduce arterial stiffness. In this context, lycopene has been shown in many studies to exert a favorable effect in patients with subclinical atherosclerosis, metabolic syndrome, hypertension, peripheral vascular disease, stroke and several other cardiovascular disorders, although the results obtained are sometimes inconsistent, which warrants further studies focusing on its bioactivity [5]. There was a study with randomized 36 statin treated CVD patients and 36 healthy volunteers in a 2:1 treatment allocation ratio to either 7 mg lycopene or placebo daily for 2 months in a double-blind trial. Post-therapy EDV responses for lycopene-treated CVD patients were similar to HVs at baseline (2% lower, 95% CI: −30% to +30%, P = 0.85), also suggesting lycopene improved endothelial function. Lycopene supplementation improves endothelial function in CVD patients on optimal secondary prevention, but not in HVs [6]. Lycopene is found in many fruits and vegetables, there is a unique situation that more than 85% of the most dominant source of lycopene in human food comes from tomatoes (*Solanum lycopersicum esculentum* Mill) and products made from tomatoes such as tomato paste, tomato sauce and tomato juice. The level of lycopene content in some tomato products varies from 8.8 μg/g in fresh tomatoes to 1200 μg/g in tomato powder. The concentration of lycopene in tomatoes varies depending on the type of tomato, season, level of maturity. The concentration of lycopene in ripe tomato juice ranges from 8600 to 9300 μg/100 g or three times higher than fresh tomatoes [7]. The study was conducted using 336 g tomato juice (350 mL) equivalent to 30 mg of lycopene with the same research time with a similar study in humans that is 3 weeks. The use of a dose of 30 mg/day based on analysis from previous studies. Studies with lycopene dose of 20 mg/day can significantly reduce total cholesterol, but not significantly in high density lipoprotein (HDL) and triglyceride (TG) [8], where studies with a lycopene dose 40 m/day show a positive correlation with total cholesterol, LDL, TG and HDL/LDL ratio, but negatively correlated with HDL [9].

This study was conducted at the Regional General Hospital Dr. H. Soewondo Kendal, because cases of dyslipidemia in employees increased significantly, in 2009 there were 67 cases and increased to 108 cases in 2012. Cases of dyslipidemia include hypercholesterolemia, hypertriglyceride, a combination of both, with Diabetes Mellitus and with other diseases. The objective of this study was to determine the effects of tomato juice supplement consumption on the lipid profile of dyslipidemic patients.

2. Materials and methods

The design of this study was a quasi-control experimental design with pre-post test. Subjects were sixty-two Kendal Hospital women employees, aged 35–50 years old, suffering from dyslipidemia but did not suffer from metabolic or degenerative diseases based on the examination of a specialist in internal medicine. Nutrition intake data was measured by 24-hour food recall method for three days in three weeks of the study. Nutritional values were analyzed using the Nutrisurvey program. Food withdrawal is carried out using a food withdrawal form through interviews conducted by nutritionists. Subjects were divided into two groups; group I as the treatment group (30 people) were given with antioxidant supplements (336 g of tomatoes per day) for 21 days and group II (32 people) as the control group. Data on total cholesterol levels, LDL cholesterol levels, HDL cholesterol levels, and triglyceride levels were measured using the chemical analysis system of vitros 300 system by clinical pathology laboratory officers of Regional Hospital Dr. H. Soewondo Kendal, accredited by the government. Measurements were taken before and after supplementation. Blood was taken at 08.00–11.00 Western Indonesian Time (WIT) and immediately checked the total cholesterol level, LDL cholesterol level, HDL cholesterol level, and triglyceride level.

Data was analyzed with Kolmogorov Smirnov test, Pearson test and Mann Whitney test. This research has been legalized by the Health Research Ethics Commission (KEPK) Faculty of Medicine, UNDIP and RSUP DR. Kariadi Semarang, with the issuance of "ETHICAL CLEARANCE" No. 329/EC/FK-RSDK/2014.

3. Results

In **Table 1**, the results showed that the profile of the study subjects did not show differences in age, BMI, cholesterol intake, Mono Unsaturated Fatty Acid (MUFA) intake, Poly Unsaturated Fatty Acid (PUFA) intake, carbohydrate intake, fiber intake, vitamin A intake, and vitamin C ($p > 0.05$). But there were differences in the percentage of energy intake and percentage fat intake ($p < 0.05$).

In **Table 2** significant relationships between energy intake ($P = 0.001$, $r = 0.501$), fat intake ($P = 0.001$, $r = 0.574$), protein intake ($P = 0.008$, $r = 335$) and carbohydrate intake ($P = 0.001$, $r = 0.446$) with total cholesterol levels were observed. There was also a significant relationship between the fat intake ($P = 0.012$, $r = 0.318$) with LDL levels and there were significant relationships between energy intake ($P = 0.005$, $r = 353$), fat intake ($P = 0.008$, $r = 336$), protein intake ($P = 0.016$, $r = 305$), and carbohydrate intake ($P = 0.013$, $r = 315$) with triglyceride levels.

Table 3 shows the lipid profile of the study subjects before the study. There were no differences between the treatment group and the control group in the total cholesterol levels and the LDL levels ($p \geq 0.05$) but, there were differences in the HDL levels and the triglyceride levels ($p < 0.05$).

Variable	Control Group (n = 32)	Treatment Group (n = 30)	Value (p)
	Average ± SD	Average ± SD	
age (th)	45.2 ± 3.4	46.3 ± 3.9	0.05[a]
imt(kg/m^2)	27 ± 2.1	27.3 ± 3.1	0.96[b]
Energy intake (% REE)	124 ± 14.4	116.5 ± 13	0.04[b]
Fat intake (% E)	36 ± 1.7	35 ± 2.7	0.04[b]
Carbohydrate intake (% E)	51 ± 1.6	50.5 ± 3.8	0.30[b]
serat intake (gr)	23.4 ± 2.4	22.8 ± 2.9	0.22[b]
Choles intake (gr)	191.2 ± 27.8	199.3 ± 42.4	0.27[b]
Mufa intake (gr)	24.6 ± 2.7	23.6 ± 2.9	0.14[b]
Pufa intake (gr)	33.4 ± 3.9	31.5 ± 3.6	0.07[b]
vit A intake (µg)	1465.6 ± 295.6	1472.6 ± 260.8	0.49[b]
vit C intake (mg)	123.4 ± 6.8	124.3 ± 9.1	0.75[b]

aMann Whitney.
bIndependent Samples t-test.

Table 1.
Characteristics of research subjects.

Variable	Total Cholesterol Level	LDL Level	TG Level
Energy intake	P = 0.001, r = 0.501	P = 0.110, r = 205	P = 0.005, r = 353
Fat intake	P = 0.001,r = 0.574	P = 0.012,r = 0.318	P = 0.008, r = 336
Protein intake	P = 0.008, r = 335	P = 0.449,r = 0.098	P = 0.016, r = 305
Carbohydrate intake	P = 0.001, r = 0.446	P = 0.325,r = 0.127	P = 0.013 r = 315

Table 2.
Relationship between food intake and research subject profiles.

Variable	Control Group (n = 32)	Treatment Group (n = 30)	Value (p)
	Average ± SD	Average ± SD	
Chol tot pre (gr/dl)	225.9 ± 19.3	231.7 ± 24.7	0.45[a]
HDL pre (gr/dl)	53.8 ± 9.6	48.7 ± 11.3	0.04[b]
LDL pre (gr/dl)	144.9 ± 29.2	150.7 ± 23.3	0.22[b]
TG pre (gr/dl)	148.9 ± 69.7	189.4 ± 79.2	0.01[a]

aMann Whitney.
bIndependent Samples t-test.

Table 3.
Lipid profile of subjects before in the treatment group and control group.

The lipid profile of the subjects after the study can be seen in **Table 4**. There is a difference between the total cholesterol and the LDL levels ($p < 0.05$) between the treatment group and the control group but no difference in the HDL levels and the triglyceride levels ($p \geq 0.05$).

The treatment results can seen in **Table 5**, there were significant differences in total cholesterol ($p = 0.001$), low lipoprotein ($p = 0.001$) and triglyceride levels ($p = 0.010$) in the treatment and the control groups. This shows that there is a significant relationship between the tomato juice supplementation and lipid profile.

Variable	Control Group (n = 32)	Treatment Group (n = 30)	Value p
	Rerata	Rerata	
Chol tot post (gr/dl)	231.3 ± 24.5	202.1 ± 23.7	0.00[a]
HDL post (gr/dl)	54.9 ± 7.5	53.1 ± 9.7	0.23[b]
LDL post (gr/dl)	144.8 ± 23.5	117.5 ± 23.05	0.00[b]
TG post (gr/dl)	156.9 ± 49.73	148.2 ± 56.05	0.55[a]

aMann Whitney.
bIndependent Samples t-test.

Table 4.
Lipid profile of subjects after in the treatment group and control group.

Variable	Treatment Group (n = 30)	Control Group (n = 32)	Value (p)
	Average ± SD	Average ± SD	
d-Chol tot (gr/dl)	-29.6 ± 15.2	5.3 ± 12.3	0.001[a]
d-HDL (gr/dl)	4.4 ± 8.9	0.83 ± 4.3	0.070[b]
d-LDL (gr/dl)	-32.5 ± 21.8	-0.8 ± 20.1	0.001[b]
d-TG (gr/dl)	-41.2 ± 67.3	8 ± 40.9	0.010[a]

a*Mann Whitney.*
[b]Independent Samples t-test.
-: decreasing value.

Table 5.
Changes in the lipid profile of the treatment and control group.

4. Discussion

The average age of the subjects is 46 years, including middle-aged adults who are at risk of fat tissue with decreased metabolism. The older a person is, the ability of LDL receptors is reduced, causing blood LDL to increase. About 9% of subjects' BMI were obese or an average of 27 kg/m2. Obese adults were more at risk than normal weight adults as they have high total cholesterol, LDL and triglyceride levels which is a major risk factor for coronary heart disease. Obesity, especially central obesity, carries a greater risk for coronary heart disease and other degenerative diseases and dyslipidemia. Excess Very Low Density Lipoprotein (VLDL) is produced in the liver in obese people and subsequently the VLDL is converted to LDL [10]. Due to that, the lipid and lipoprotein profiles of obese people are changed with increasing triglycerides and decreasing HDL [11].

Most of the subjects (90.3%) have high energy intake at the average of 2300 kcal. High energy intake results in fat accumulation, especially triglycerides. This will increase VLDL and LDL in blood which will cause an increase in total cholesterol. The fat intake of the study subjects exceeded the nutritional adequacy rate (25–30%) i.e. the average was 34.49% in the treatment group and 35.66% in the control group. When a person consumes more fatty food, fat and triglyceride levels in the body will increase. This will increase blood VLDL and LDL [12].

Significant relationships between energy intake (P = 0.001, r = 0.501), fat intake (P = 0.001, r = 0.574), protein intake (P = 0.008, r = 335) and carbohydrate intake (P = 0.001, r = 0.446) with total cholesterol levels were observed. There was a significant relationship between fat intake (P = 0.012, r = 0.318) with LDL levels. There were significant relationships between energy intake (P = 0.005, r = 353),

fat intake (P = 0.008, r = 336), protein intake (P = 0.016, r = 305) and carbohydrate intake (P = 0.013, r = 315) with triglyceride levels, which is in line with this research. When compared with a higher-carbohydrate Dietary Approaches to Stop Hypertension (DASH) diet, DASH-type diet with higher protein lowered LDL by 3.3 mg/dL, and triglycerides by 16 mg/dL. Compared with a higher-carbohydrate DASH diet, DASH-type diet with higher unsaturated fat lowered triglycerides by 10 mg/dL [13]. In a meta-analysis of 60 randomized controlled feeding trials, consumption of 1% of calories lowered triglycerides [14].

In a meta-analysis of four prospective cohort studies involving nearly 140,000 subjects, including updated analyses from the two largest studies, a 2 percent increase in energy intake from trans fatty acids was associated with a 23 percent increase in the incidence of CHD (pooled relative risk, 1.23; 95 percent confidence interval, 1.11 to 1.37; P < 0.001) [15]. In meta-analyses of prospective cohort studies, greater consumption of refined complex carbohydrates, starches, and sugars, as assessed by glycemic index or load, was associated with significantly higher risk of CHD and DM. When the highest category (total sedentary time) was compared with the lowest category (short sedentary time), risk of CHD was 36% greater (glycemic load: RR, 1.36; 95% CI, 1.13–1.63) and risk of Diabetes Mellitus was 40% greater (glycemic index: RR, 1.40; 95% CI, 1.23–1.59) [16, 17].

When the highest category was compared with the lowest category, dietary Linoleic Acid (LA) was associated with a 15% lower risk of CHD events (pooled RR, 0.85; 95% confidence intervals, 0.78–0.92; I(2) = 35.5%) and a 21% lower risk of CHD deaths (pooled RR, 0.79; 95% confidence intervals, 0.71–0.89; I(2) = 0.0%). A 5% of energy increment in LA intake replacing energy from saturated fat intake was associated with a 9% lower risk of CHD events (RR, 0.91; 95% confidence intervals, 0.87–0.96) and a 13% lower risk of CHD deaths (RR, 0.87; 95% confidence intervals, 0.82–0.94) [18].

The association suggested that replacing SFA with PUFA rather than MUFA or carbohydrates prevents CHD through various intakes. In comparison, in an analysis of individual levels collected from 11 prospective cohort studies, the specific exchange of PUFA consumption at SFA sites was associated with a lower risk of CHD, with a 13% lower risk for each 5% energy exchange (RR, 0.87; 95% CI, 0.70–0.97 [19].

These findings provided evidence that consuming PUFA as a substitute for SFA reduces the incidence of CHD in RCTs. This also showed that instead of trying to reduce PUFA consumption, a shift towards a larger PUFA population as a substitute for SFA will significantly reduce the level of CHD [20].

The replacement of animal fats, including dairy fat, with vegetable sources of fats and PUFAs may reduce risk of CVD. whereas the 5% energy intake substitution of other animal fat with dairy fat was associated with 6% increased CVD risk (RR: 1.06; 95% CI: 1.02, 1.09) [21].

There were significant differences in total cholesterol (p = 0.001), low lipoprotein (p = 0.001) and triglyceride levels (p = 0.010) in the treatment and control groups. This shows that supplementation of 336 g (350 mL) tomato juice per day for 21 days in women with dyslipidemia with minimal risk can reduce total cholesterol levels by 29.6 g/dL, LDL levels by 32.5 g/dL, triglyceride levels by 41.4 g/dL.

The results of this study are in line with research by Mozaffarian *et al.* [20] which states that these findings are consistent with a meta-analysis of RCTs in which increased PUFA consumption in place of SFAs reduced CHD events, with 10% lower risk for each 5% energy exchange (RR, 0.90; 95% CI, 0.83–0.97.

Among Chinese adults, a higher level of fruit consumption is associated with a lower risk of major cardiovascular disease [22]. Several studies have examined the

relationship between antioxidant intake and lipid peroxidation to try to determine which antioxidants play a role in preventing cardiovascular disease. The hydrocarbon carotenoids, including β-carotene and lycopene, are transported primarily in LDL, which puts them in prime position to protect LDL from oxidation [4].

Lycopene may have a cholesterol synthesis-inhibiting effect and may enhance LDL degradation. Available evidence suggests that intimal wall thickness and risk of myocardial infarction are reduced in persons with higher adipose tissue concentrations of lycopene [23].

Treatment with lycopene increased the ejection fraction (EF) from 45.2 ± 3.12 to 51.1 ± 4.63, and it decreased the left ventricular at end-diastole diameter (LVEDd) from 6.52 ± 0.37 mm to 6.18 ± 0.41 mm and the left ventricular at end-systole diameter (LVESd) from 4.29 ± 0.63 to 3.94 ± 0.37 at 28 days post-myocardial infarction. Lycopene attenuated the MI-induced increase in MMP-9 and type I collagen expression, and inhibited p38 activation. Moreover, lycopene decreased the collagen volume fraction in the peri-infarcted zone. The data indicated that lycopene improved the cardiac function and ventricular remodeling by inhibiting p38 activation and MMP-9 expression [24]. Lycopene can suppress VSMCs proliferation, due to inhibiting G1 phase cells entry into the S phase of the cell cycle, related to its antioxidative effect [25], and synthesis of oxidized LDL may be impaired by lycopene [26]. Loana *et al.* [5] reviewed the importance of lycopene in improving vascular function and in the primary and secondary prevention of cardiovascular disorders. The effects shown by lycopene in view of cardiovascular health consist of general antioxidants and anti-inflammatory, antiplatelet, anti-apoptotic and antihypertensive properties, ability to improve endothelial function, metabolic profile and ventricular remodeling, reduction in arterial stiffness as well as reduction in atherosclerotic plaque size. Lycopene exerts favorable effects in patients with subclinical atherosclerosis, metabolic syndrome, hypertension, peripheral vascular disease, and several other cardiovascular disorders, but sometimes conflicting results were obtained. Clearly, more and better-designed studies will be necessary to improve our understanding of the positive effects of lycopene on vascular health and to elucidate the involved mechanisms on a molecular level. Future cardiovascular disease prevention strategies might include lycopene-enriched products, lycopene supplementation and new combinations including lycopene. Future studies should be focused on dietary lycopene and its synergistic effects with other dietary components in different study populations, with elevated cardiovascular risk, are highly warranted which might enable development of functional foods useful in prevention and complementary treatment of cardiovascular disorders [4].

5. Conclusion

There is a significant relationship between energy intake, fat intake, protein intake and carbohydrate intake with total cholesterol levels. So, there is a significant relationship between fat intake with LDL levels and there is a significant relationship between energy intake, fat intake, protein intake and carbohydrate intake with triglyceride levels. About 53.3% of the treatment group subjects had total cholesterol levels normal (<200 mg/dL) after the study and 20% of the treatment group subjects had their LDL levels normal (<100 mg/dL) after the study. Supplementation of 336 g (350 mL) tomato juice per day for 21 days in women with dyslipidemia with minimal risk can reduce total cholesterol levels by 29.6 g/dL, LDL levels by 32.5 g/dL, and triglyceride levels by 41.4 g/dL.

Author details

Sugini
Nutrition Installation of Kendal District General Hospital, Central Java Province, Indonesia

*Address all correspondence to: suginigizi@gmail.com

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Chapter 12

Utility and Appropriate Use of PCSK9 Inhibitors in the Current Era

Aniruddha Singh, Travis Huffman and Megan Smith

Abstract

Atherosclerotic cardiovascular diseases (ASCVD) remain the number one cause of death and morbidity in the country. Elevated cholesterol/hyperlipidemia has been considered one of the major risk factors for ASCVD. Statins have been the main stay therapy for treating hyperlipidemia achieving remarkable clinical benefits; however; its inability to achieve the desired reduction in the low density lipoprotein cholesterol (LDL) in some people and the disabling side effects from it in others, has led to a search for an alternative therapy. One of the groundbreaking inventions in this field has been the advent of the proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i). These agents have similar efficacy to high intensity statins and at the same time are tolerated well with a low incidence of side effects. Based on the results from multiple large scale clinical outcome trials, this class of LDL-C lowering agents has been recommended as appropriate second-line agents, or as an alternative therapy in cases of significant statin intolerance, for patients with established ASCVD and suboptimal LDL levels. As evidence supporting their efficacy and safety continues to mount, use of PCSK9i is likely to keep expanding in the current ASCVD population. In this chapter we discuss the advent of PCSK9i, their clinical utility, and their appropriate use keeping in view the high drug cost and other barriers in prescribing them.

Keywords: proprotein convertase subtilisin/kexin type 9 (PCSK9i), hyperlipidemia, ASCVD, LDL-C, statins, evolocumab, alirocumab

1. Introduction

Hyperlipidemia is a well-known and established cardiovascular risk factor, with a global prevalence of approximately 39% per a World Health Organization report from 2011 [1]. Cholesterol lowering therapy, specifically low-density lipoprotein (LDL) lowering therapy, has been shown to significantly reduce the prevalence of atherosclerotic cardiovascular disease (ASCVD). The management of hyperlipidemia has evolved greatly over the last thirty years. Since the development of commercially available statins in the 1980's, this class of medication has become the hallmark for treatment of hyperlipidemia, with multiple adjunct therapies emerging over the past decade. And while statins are substantially efficacious in lowering LDL-C, a portion of patients are not able to achieve their goal LDL-C on statins alone. Another subset of patients develop statin induced side effects which either

limits up titration of statin therapy, or in some patients, even prevents the use of a small dose of statins. In order to obtain optimal ASCVD risk reduction, further lowering the LDL-C has been achieved with the use of ezetimibe, as well as the use of proprotein convertase subtilisin/kexin type 9-inhibitors (PCSK9i).

The PCSK9i: evolocumab and alirocumab, have shown promising results over the last several years, not only in major randomized landmark trials but also in clinical practice. These agents are one of the most efficacious antihyperlipidemic therapies and have shown to achieve a reduction in low density lipoprotein (LDL) by 50–65% [2, 3]. Two large outcomes trials [4, 5] have demonstrated that both evolocumab and alirocumab are effective in reducing major adverse cardiac events in high risk atherosclerotic cardiovascular disease (ASCVD) conditions. Most recent multi-society guidelines are endorsing the use of PCSK9i in patients at very high cardiovascular risk [6], which includes patients with familial hypercholesterolemia. A joint consensus statement from the European Society of Cardiology and European Atherosclerosis Society suggested that PCSK9i could be considered in patients with clinical ASCVD treated with maximal tolerated statin therapy and/or ezetimibe who have continued to have LDL-C > 100 mg/dL [7]. Despite all the available data supporting its efficacy and clinical benefits, the cost-effectiveness and economic value of PCSK9 inhibitors has been reported to be 'low value' as measured in quality adjusted age years. [6]. The guidelines have also felt that the economic value of PCSK9i would be improved by restricting its use to patients at high risk of ASCVD events, specifically initiating PCSK9i in high risk patients with LDL-C values >120, despite optimal lipid lowering therapy. In this article we describe the clinical use of PCSK9i in the current era and its appropriate use.

2. Mechanism of action

The proprotein convertase subtilisin/kexin-9 (PCSK9) is a serine protease, which has been found to be integral in the regulation of LDL-C plasma concentrations. The LDL receptors (LDL-R) present on hepatocytes are responsible for binding circulating LDL-C and removing them from plasma. In the absence of PCKS9, the LDL-C/LDL-R complex enters hepatocytes within the endosome and dissociates into LDL-C and LDL-R as a result of the acidic pH present in the endosome. The LDL-R is then recycled back to the hepatocyte surface, making it available to bind more LDL-C, thereby lowering the serum LDL-C concentration. In the presence of PCSK9, however, the LDL-C/LDL-R complex does not dissociate within the endosome, and the entire complex is marked for lysosomal degradation. Without LDL-R recycling, less LDL-C is removed from circulation, resulting in higher LDL-C plasma concentrations [8].

The clinical implications of PCSK9 have been demonstrated in both loss of function and gain of function mutations. A gain of function mutation was first discovered in two French families with familial hypercholesterolemia that was not associated with LDLR or APOB mutations [9]. The mutated allele created an overexpression of PCSK9, and subsequently elevated plasma LDL-C levels, with sequelae of significant hypercholesterolemia including tendinous xanthomas and risk of premature ASCVD in the fourth and fifth decade. While gain of mutation functions have been discovered in other cohorts in Utah, Norway and the United Kingdom, familial hypercholesterolemia secondary to PCKS9 gain of function mutations is uniquely rare [8]. However, this discovery provided insight to PCSK9 activity, and that overexpression of this protein results in excessive LDL-C in vivo. This discovery also provided the third locus for autosomal dominant familial

hypercholesterolemia inheritance, adding to the already known LDL-R and APOB mutations [9].

Conversely, loss of function mutations of PCKS9 have been shown to significantly reduce circulating LDL levels, which sparked interest in accumulating data on how this translated to reduced ASCVD risk. One study discovered that loss of function mutations in one population was present in 2.3% of black patients, and 3.2% of white patients. The loss of PCKS9 function resulted in a 28 percent mean reduction of LDL, and an 88 percent reduction in ASCVD risk in the black population, and a 15 percent mean reduction in LDL and a 47 percent reduction in ASCVD risk in the white populations [10]. This review also supported the idea that loss of PCSK9 function would not impact viability, as both populations in this review had intact reproductive or neurologic function. This was further corroborated in PSCK9 knockout mice, in which no PCKS9 function resulted in exceedingly low LDL-C levels [11, 12]. These findings suggest that PCKS9 function is not vital for life, and complete inhibition of this proteinase would be well tolerated in humans, further sparking the search to create a mechanism in which we could inhibit PCKS9 function pharmaceutically.

Currently, two monoclonal against PCSK9 are available: alirocumab and evolocumab, which bind with a 1: 1 ratio to circulating PCSK9. Once the antibody binds to PCSK9, PCSK9 is unable to attach to LDL receptors which in turn inhibits the receptors' degradation. This leads to an increased expression of LDL receptors on hepatocytes, leading subsequently to rapid clearance of LDL particles [13].

3. Approved indication

The Food and Drug Administration (FDA) has approved alirocumab and evolocumab as "an adjunct to diet and maximally tolerated statin therapy for treatment of adults with heterozygous Familial Hypercholesterolemia (FH) or clinical ASCVD who require additional lowering of LDL-C." Evolocumab is also indicated for treatment of homozygous FH and, based on the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) trial, "to reduce the risk of myocardial infarction (MI), stroke, and coronary revascularization in adults with established CVD."

4. Dosing and adverse effects

Alirocumab in the doses of either 75 mg or 150 mg could either be given subcutaneously every 2 weeks, or as a monthly 300 mg subcutaneous injection. LCL-C levels decrease around 45% from the 75 mg dose, and LCL-C levels decrease approximately 55–60% with the 150 mg dose.

Evolocumab also could be either given in a dose of 140 mg subcutaneous injections every 2 weeks, or as a 420 mg subcutaneous injection monthly. Both doses lower LDL-C approximately 55–60%. Besides lowering LDL, both alirocumab and evolocumab achieve mild lowering of triglycerides by 10–15% range. A modest increase in HDL cholesterol by 5–10% has been noted as well.

5. Adverse reactions

Trials involving PCKS9 inhibitors have included patients that were both tolerant and intolerant to statin therapy, providing information on their safety profile.

Serious adverse reactions attributed directly to drug injection have been rarely reported, with <10% of patients requiring drug discontinuation during separate study trials [14, 15].

With both available PCKS9i injectable solutions, the most reported adverse effect has been injection site reaction, which occurs in approximately >5% of patients, including injection site allergic reactions. Additional adverse reactions that have been reported by both study data and real-world use databases include upper respiratory infections, nasopharyngitis, myalgias, back pain and neurocognitive events including memory impairment and confusion [14, 15].

In regard to myalgia and muscle symptoms, evolocumab was studied head to head against ezetimibe in the GAUSS-3 trial [14]. This trial examined patient who were intolerant to statins due to muscle symptoms, and randomly assigned patients to either evolocumab or ezetimibe. In this trial population, drug discontinuation due to muscle symptoms occurred in 6.8% of ezetimibe patients and 0.7% of evolocumab patients. However, more patients in the evolucumab arm, 2.8% had elevations in CK levels, compared to 1.4% of patients in the ezetimibe arm.

The only absolute contraindication to PCKS9 inhibitor use is a history of hypersensitivity reaction to prior PCKS9 injections, with limited data available to determine whether allergenic cross-reactivity exists between the two available PCKS9 formulations.

6. Available scientific evidence

The FOURIER trial (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) tested clinical outcomes when evolocumab was added to maximum statin therapy in ASCVD patients [16]. A total of 27,564 patients were randomized to either evolocumab (either 140 mg every 2 weeks or 420 mg monthly) or placebo. Patients who were treated with evolocumab had close to 60% LCL-C lowering. After a follow up period of 24 months, the combined endpoint of cardiovascular mortality, myocardial infarction, stroke, hospitalization related to angina, or revascularization was seen in 9.8% of patients in the evolocumab group compared to 11.3% in the placebo group.

The ODYSSEY OUTCOMES trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) evaluated outcomes in patients treated with alirocumab after acute coronary syndrome who were already on maximally tolerated statins [17]. A total of 18,924 patients were randomized to either alirocumab or placebo. The composite endpoint of CV death, nonfatal myocardial infarction, fatal and nonfatal stroke, or hospitalization due to angina occurred less the alirocumab arm compared to placebo (9.5% Vs. 11.1%, $p = 0.003$).

The GLAGOV (Global Assessment of Plaque Regression With a PCSK9 Antibody as Measured by Intravascular Ultrasound) trial was designed to study the effect of using PCSK9 inhibition on atherosclerotic plaque burden and regression [18]. Over a period of 18 months a total of 968 patients with ASCVD were treated with either evolocumab or placebo. LDL-C levels were significantly lower in the evolocumab group compared to the placebo group. Atheroma volume was assessed using intravascular ultrasound, which demonstrated larger reduction in the evolocumab group compared to placebo.

The ODYSSEY ALTERNATIVE trial assessed the performance of alirocumab against ezetimibe and atorvastatin in patients with statin associated skeletal muscle effects [19]. Patients were randomized in a 2:2:1 fashion to either alirocumab 75 mg subcutaneously Q2W, ezetimibe 10 mg daily, or atorvastatin 20 mg daily.

Alirocumab significantly reduced LDL-C at 24 weeks compared with ezetimibe; % decrease was 45% vs. 14.6%, difference 30.4, $p < 0.0001$. Muscle-related side effects were lower in the alirocumab arm (32.5%) compared with the ezetimibe (41.1%, $p = 0.096$) and atorvastatin arms (46%, $p = 0.042$). Alirocumab was found to be superior to ezetimibe in lowering LDL-C levels and achieving target levels in patients with statin intolerance, with a lower risk of muscle-related side effects.

The GAUSS-2 (Global Achievement After Utilizing an Anti-PCSK9 Antibody in Statin Intolerant Subjects-2) trial assessed primarily the safety of evolocumab in patients with statin-associated muscle side effects [20]. It was a double-blind study in which patients were randomized to either evolocumab or ezetimibe. Superior efficacy and no muscle-related adverse events requiring discontinuation was seen in patients treated with evolocumab.

7. Appropriate use

Based on the results from multiple large scale clinical outcome trials of PCSK9i, this class of LDL-C lowering agents has been recommended as appropriate second-line agents, or as an alternative therapy in cases of significant statin intolerance, for patients with established ASCVD and suboptimal LDL levels. As evidence supporting their efficacy and safety continues to mount, use of PCSK9i is likely to keep expanding in the current ASCVD population.

The ACC/AHA multi-society Cholesterol Clinical Practical Guidelines released in late 2018 give a class I recommendation for PSCK9i in patients with clinical ASCVD and very high risk (VHR) for future ASCVD events who are on maximally tolerated statin therapy and ezetimibe [6]. There is also a class IIa recommendation for VHR patients with ASCVD taking maximally tolerated statin therapy and ezetimibe with LDL-C ≥ 70 mg/dL (≥1.8 mmol/L) or a non-HDL-C level ≥ 100 mg/dL (≥2.6 mmol/L) to consider PCSK9i. Criteria to be considered VHR include major ASCVD events such as acute coronary syndrome within the past 12 months, history of myocardial infarction, history of embolic stroke, and symptomatic peripheral arterial disease, as well as high-risk conditions such as age ≥ 65 years old, heterozygous familial hypercholesterolemia, history of primary coronary artery bypass surgery or percutaneous coronary intervention, diabetes mellitus, hypertension, chronic kidney disease stage III or worse, ongoing tobacco use, persistent elevated LDL-C ≥ 100 mg/dL (≥2.6 mmol/L) despite maximally tolerated statin therapy and ezetimibe, and history of congestive heart failure. Regarding primary prevention, patients with heterozygous familial hypercholesterolemia aged 30 to 75 years old with an LDL-C ≥ 100 mg/dL (≥2.6 mmol/L) while taking maximally tolerated statin and ezetimibe therapy and patients aged 40 to 75 years old with a baseline LDL-C ≥ 220 mg/dL (≥5.7 mmol/L) and have an on-treatment LDL-C ≥ 130 mg/dL (≥3.4 mmol/L) while taking maximally tolerated statin and ezetimibe, the addition of a PCSK9i is given a class IIb recommendation.

Despite strong multi-societal recommendations, uptake rates for PCSK9i have lagged. A study looked at electronic health record data to characterize use of PCSK9i, in addition to standard therapies [21]. Data were obtained from 18 health systems within the National Patient-Centered Clinical Research Network using a common data model. Out of more than 17.5 million adults, 3.6 million met study criteria. Approximately half of patients had been prescribed lipid-lowering medication but <1% were prescribed PCSK9i. A trend towards increased PCSK9i prescription over time was seen for patients with ASCVD but not for those with dyslipidemia. PCSK9i, which effectively lower LDL cholesterol, had low use during this surveillance period.

Cost, or perceived expense, has been another barrier limiting patient access to PCSK9i use. High co-pays have been shown to lower access to PCSK9i, despite Medicare and other third-party payer coverage for PCSK9i [22]. Cost-effective analyses are essential in navigating which scenarios are prudent for treatment. Prior to October 2018, most cost-effectiveness studies concluded that PCSK9i were not cost-effective with the accepted threshold of $100,000 per quality-adjusted life-year (QALY) gained and cost of treatment estimated around $14,000 a year [23, 24]. However, in October 2018, the list price of evolocumab was decreased by 60% to $5850 in the United States, in an effort to lower copays and improve patient access. This dropped the ratios below the threshold of $50,000 per QALY gained and meeting accepted cost-effectiveness benchmarks [25]. Reducing cost can improve access to PCSK9i, however, prior authorization requirements, lack of insurance approval, and unfamiliarity amongst healthcare providers with this relatively new class of drug remain problematic.

8. Conclusion

PCSK9i have emerged as a breakthrough antihyperlipidemic therapy in the current era, where ASCVD is the leading cause for morbidity and mortality. PCSK9i are efficacious and safe. Current literature on their suboptimal use in real-world settings indicates that a large proportion of these patients could benefit from more aggressive treatment with this class of lipid lowering therapy. The cost-effectiveness of PCSK9i can be improved by restricting its use in patients with increased risk of ASCVD, who derived more benefit in the PCSK9i outcome trials. Also, the most recent AHA/ACC multi-society guidelines on the management of hyperlipidemia gave a class 1 recommendation to adding ezetimibe to maximally tolerated statin therapy for secondary prevention prior to initiation of PCSK9 inhibitors. This would also help triage PCSK9i use in people who are highest risk which in turn would improve its cost effectiveness.

Author details

Aniruddha Singh*, Travis Huffman and Megan Smith
Western Kentucky Heart and Lung Research Foundation, The Medical Center, University of Kentucky College of Medicine, Bowling Green, KY, USA

*Address all correspondence to: aniruddha.singh@uky.edu

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Edited by Wilbert S. Aronow

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Published in London, UK

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ISBN 978-1-83968-509-5
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