A new perspective on the development of cholesterol-lowering products
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1. Introduction

1.1. Health impact of cardiovascular disease (CVD)

Cardiovascular disease (CVD) is the principal cause of death worldwide, representing nearly 30% of the annual global mortality and 10% of global health burden [1]. The current status of CVD is now on international scale; which can be considered as the commonest chronic illness in both developed and developing countries, causing the most deaths and the greatest impact on morbidity [2]. In 2006, CVD was the leading cause of death for Canadians, representing 30% of all deaths [3]. A total number of 1.3 million Canadians are diagnosed having heart disease accounting for 5% among those above 12 years and 23% at 75 years and older. The increased rate of obesity and diabetes combined with further aging of the population will likely lead to an increase in the number of people with CVD in the future. This will compromise the health of Canadians, put a strain on the health care system, and have a significant economic impact on Canada [4]. Similarly, over the past five decades the prevalence of CVD has steadily increased in economically developing countries [5]. These countries will account for 76% of an estimated 25 million death due to CVD in 2020 [6]. On an international basis, by 2020 CVD will reach nearly epidemic proportions and become the cause of more deaths, disability and economic loss than any others group of diseases. The number of fatalities by CVD projected to increase to over 20 million a year by 2020 and over 24 million a year by 2030 [7]. Apparently, understanding the aetiology of CVD and accordingly develop preventive and therapeutic approaches to address this health threat continues to be critically important in the next decades although significant achievements have been made in the past decades.
2. Risk factors of CVD

The aetiology of CVD is multifactorial, complex and still not completely understood. However, there is now a general agreement that elevated total cholesterol, LDL-cholesterol and triacylglycerol levels, low HDL-cholesterol concentrations, smoking, high blood pressure, hyperglycemia and diabetes are all risk factors of CVD. Physical inactivity, obesity, diet and low socio-economic status are thought to be predisposing risk factors which work, at least in part, as promoter on other risk factors. These factors predispose to develop syndrome X or metabolic syndrome, which is characterised by obesity, hypertension, dyslipoproteinaemia, and disturbed glucose tolerance [8]. Some other factors such as elevated prothrombotic factors, markers of inflammation, elevated homocysteine, elevated lipoprotein (a) and some psychological factors show associations with CVD [6]. Nevertheless, the aetiology of CVD is far from clear [9], and most factors are unmodifiable while others can be modified through the change of diet habits and lifestyles.

2.1. Unmodifiable risk factors

2.1.1. Ageing

Cardiovascular diseases, such as atherosclerosis, coronary heart disease and resultant heart failure reach epidemic proportions among older persons. Aging leads to arterial stiffening that results in aortic dilation and wall thickening along with increased collagen level. Potential age-associated changes in the tissue levels or responses to growth factors, catecholamines, angiotensin II, endothelin tumor growth factors β (TGF β) or fibroblast growth factors influences myocardial or vascular cells. Deficit in myocardial beta adrenergic receptor signalling, decline in omega-3 polyunsaturated fatty acids and increased reactive oxygen species generation occur with aging, which enhances Ca\(^{2+}\) influx [10]. The clinical manifestations and prognosis of CVD and resultant heart failure worsen with ageing. Over 83 percent of people who die of coronary heart disease are 65 or older. Thus, age, per se, is the major risk factor for CVD [10].

2.1.2. Gender

There is a marked difference in CVD risk between sexes [11]. Incidence of CVD was approximately 3-fold and mortality about 5-fold greater in men than in women [12]. Among middle-aged people, incidence of coronary heart disease is 2 to 5 times more in men than in women, and this sex ratio varies between populations. The role of major risk factors such as lipid abnormalities, high blood pressure, smoking, obesity and diabetes in the development of CVD is well established among men (Jousilahti, Vartiainen et al. 1999). In women, significant shift in their physiological function and health profiles occurs during and the postmenopausal. When entering into middle ages, women tend to have lower LDL cholesterol and higher HDL cholesterol values than men of similar age. Following menopause, total cholesterol, LDL cholesterol and triglycerides levels increases while HDL levels remain unchanged or decrease.
slightly. These alterations in lipid values are thought to be related in part to loss of protective effects of estrogen [13].

2.1.3. Heredity (including race)

The prevalence of CVD considerably varies by race/ethnicity. Genetic predispositions are a result of gene mutations, which alter the biological function expressed by the original genes (polymorphisms) and increases an individual risk for the disease. Several polymorphism and linkage markers have been identified as being correlated to the onset of CVD. For example, M235T polymorphism of the angiotensinogen gene is linked to hypertension and later to the development of CVD [14]. In many developed countries, racial and ethnic minorities bear disproportionate burden of heart disease. Significant differences in socioeconomic status and conventional heart disease risk factors exist among racial/ethnic groups in the United States, Canada and United Kingdom (Ludwig, Ebbeling et al. 2002). The rate of heart disease among the racial and ethnic group in the United States vary widely, with African Americans having rates a half to three fold greater than Asians, depending on the gender [15]. It is also well documented that the prevalence of CVD is higher in several minority populations (Hispanics and African Americans) in comparison to whites. [16]. However, these differences do not fully account for the observed disparities in disease prevalence, suggesting the presence of other biological factors [17].

2.2. Modifiable risk factors

2.2.1. Obesity

Obesity is an independent risk factor for CVD. It is a chronic metabolic disorder associated with increased morbidity and mortality. Obesity may affect atherosclerosis through many risk factors such as dyslipidemia, hypertension, glucose intolerance, and increased chronic inflammatory and prothrombotic state. Obesity causes a variety of adaptations/alterations in cardiac structure and function due to excessive adipose tissue accumulation, even in the absence of comorbidities [18]. Thus, it increases cardiac workload that leads to heart failure, coronary heart disease, sudden cardiac death, and arterial fibrillation [19]. In many cases, these events result in mortality and morbidity. By favourably modifying blood lipids, in particular LDL cholesterol, lowering blood pressure, controlling blood sugar, decreasing proinflammatory cytokines and adhesion molecules, weight loss may prevent the progression of atherosclerosis or the occurrence of acute coronary heart events in the obese high-risk populations [18].

2.2.2. High blood cholesterol

A high concentration of serum cholesterol is a major risk factor for coronary heart disease [20]. The relationship between abnormal plasma cholesterol fractions and increased CVD risk was described 60 years ago [21]. The excessive cholesterol, especially cholesterol transported/carried by low density lipoproteins that contain protein apolipoprotein (apo) B100 contributes to the formation of atherosclerotic plaques in arteries. Accordingly, cholesterol that is carried
by LDL particles is called “bad cholesterol”. by contrast, HDL cholesterol which transports esterified cholesterol from the periphery to the liver is considered more cardioprotective and sometimes referred to as “good cholesterol” [22]. The ratio of LDL to HDL cholesterol is more important than LDL or HDL cholesterol concentration and has been widely used to evaluate susceptibility to the development of heart disease. For a healthy person, it is recommended to maintain the LDL/HDL ratio below 3.5.

2.2.3. High blood pressure

Hypertension is a highly prevalent major contributor to atherosclerotic cardiovascular disease. It accelerates atherogenesis, imparting a 2- to 3-fold coronary heart disease (CHD) and lethal sequel [23]. In most cases, hypertension results from excessive vasoconstriction of the small arterioles throughout the body, raising the diastolic pressure. Because of high peripheral resistance, the heart needs to generate more force to overcome the resistance created by the constricted arterioles and supply adequate blood to the tissues, which leads to a compensatory rise in systolic blood pressure. This excess of systolic and diastolic blood pressures causes excessive vasoconstriction. Thus, increased levels of systolic and diastolic blood pressure are associated with an increased risk of CVD events [24]. When high blood pressures co-exist with obesity, smoking, hypercholesterolemia, and/or diabetes, the risk of heart attack and stroke increases several times.

2.2.4. Physical inactivity

Sedentary lifestyle is associated with almost twice the risk of developing coronary heart disease compared with their active counterparts. Regular physical activity plays a crucial role in the prevention of CVD. High levels of physical activity are associated with substantial reductions in CVD risk, and total mortality decreases by 20% to 30% for the increase of every 1000 kcal/wk of energy expenditure resulting from physical activity [25]. Regular exercise has a favourable effect on many of the established risk factors of CVD. Exercise promotes weight reduction, reduce blood pressure, “bad” cholesterol (LDL level), and total cholesterol, and can raise the “good” cholesterol (HDL) [26].

2.2.5. Diabetes mellitus

Diabetes has long been recognized to be an independent risk factor for CVD. Type-1 diabetes or type-2 diabetes is at high risk for several cardiovascular disorders: coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy, and congestive heart failure. Closely linked to type-2 diabetes are several metabolic risk factors such as hypertension, atherogenic dyslipidemia which is associated with insulin resistance that relates to coronary heart disease. Cardiovascular complications are now the leading causes of illness and death in the diabetic patient. The incidence of diabetes rises with advancing age, obese and overweight persons and in the populations (race/ethnicity) who are particularly susceptible to diabetes [27].
2.2.6. Tobacco smoke

Smoking nearly doubles the risk of heart disease. Smoking acts synergistically with other risk factors, substantially increasing the risk of CVD [28]. The exact toxic components of cigarette smoke and the mechanisms involved in smoking-related cardiovascular dysfunction are not clearly elucidated, but smoking increases inflammation, thrombosis, and oxidation of LDL cholesterol. Smokers have significantly higher serum total cholesterol, LDL cholesterol and triacylglycerol levels, while having lower blood concentration of HDL cholesterol than non-smokers [29]. Cigarette smoke exposure increases oxidative stress as a potential mechanism for initiating cardiovascular dysfunction. Cigarette smoke exposure decreases the plasma activity of paraoxonase, an enzyme that protects against LDL oxidation. Smoking is also found to be an independent predictor of new coronary lesion formation and thrombosis [30].

In spite of identification of many unmodifiable and modifiable risk factors, there are still several paradoxes in the pathogenesis of CVD that cannot be sufficiently explained; mortality from CVD is relatively low despite a high intake of saturated fatty acids [31] or a high incidence of CVD without having the expected risk indicators [32]. CVD mortality rate in urban populations is higher compared with rural populations despite a very low fat intake [33]. All these paradoxes support the assumption that some important factors in the aetiology of CVD are currently unknown. However, it has been well established that elevated blood total cholesterol, especially LDL cholesterol levels is one of the primary risk factors that contribute to the development of atherosclerosis and ultimately CVD. Therefore, in the following sections, we focus mainly on the atherosclerosis, cholesterol metabolism and homeostasis, benefits versus side effects of current cholesterol-lowering products, and our perspectives on the development of future cholesterol-lowering products.

3. Atherosclerosis and CVD

Atherosclerosis is a disease of the arterial wall that is characterized by cholesterol accumulation and culminates in potentially life-threatening conditions such as heart attack, stroke and angina. The build-up of cholesterol in the walls of arteries is a hallmark of atherosclerosis. However, the process starts is poorly understood. The atherogenic process starts at an early age with the deposition in blood vessel walls of lipids such as cholesterol, derived from lipoproteins circulating in the bloodstream, which leads to the formation of the characteristic ‘fatty streaks’. Inflammatory white blood cells congregate at these damaged areas through their interaction with adhesion molecules expressed by cells in the endothelial layer, which lines the inside of blood vessels. This event then sets off a cascade of inflammatory process and further lipid deposition, leading eventually to full blown atherosclerosis with plaque formation in the artery wall. Atherosclerosis is thus viewed as a chronic inflammatory disease of the blood vessel wall [34], [35]. Oxidized LDL contributes to atherogenesis and is an early event of atherosclerosis. When LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalized by macrophages by means of the scavenger receptors on the surfaces of these cells. The internalization leads to the formation of lipid peroxides and
facilitates the accumulation of cholesterol esters, resulting in the formation of foam cells. As the fatty streak progresses, smooth muscle cells (not normally present in the subendothelial space) migrate from the media to the subendothelial space where they proliferate and produce connective tissue to form a fibrous cap, which represents the second phase of atherosclerosis. Finally, complicated lesions occur, which can manifest calcification, hemorrhage, ulceration and thrombosis [35, 36]. All these changes and events lead to the hardening and thickening of artery wall, reducing or blocking blood flow. Atherosclerosis is a silent and asymptomatic disease until complications arise with thrombosis and occurrence of clinical symptoms [37]. The clinical manifestation of atherosclerotic plaque formation is acute vascular occlusion due to the formation of a thrombus or clot which can lead to ischemia of vital organs, such as heart causing myocardial infarction, brain resulting in strokes and lower extremities causing peripheral artery disease. Oxidized LDL contributes to atherothrombosis by inducing endothelial cell apoptosis, and thus plaque erosion, by impairing the anticoagulant balance in endothelium, stimulating tissue factor production by smooth muscle cells, and inducing apoptosis in macrophages [38]. It is reasonably thinking that maintaining a healthy cholesterol or lipid profile is critically important to the health cardiovascular system.

4. Cholesterol homeostasis

Cholesterol, the characteristic steroid alcohol of animal tissues, performs a number of essential functions in the body. For example, cholesterol is a structural component of cell membranes and modulates cell membrane fluidity; cholesterol is a precursor of bile acids, steroid hormones and vitamin D. It is therefore of critical importance that the cells of the body be assured a continuous supply of cholesterol. To meet this need, a complex series of transport, biosynthetic and regulatory mechanism has evolved. The liver plays a central role in the regulation of the body’s cholesterol homeostasis. For example, cholesterol enters the liver’s cholesterol pool from a number of sources including dietary cholesterol, as well as cholesterol synthesized de novo by extra hepatic tissues as well as by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile or it can be converted to bile acids that are secreted in bile into the intestinal lumen.

Cholesterol, similar with other lipids (triacylglycerols and phospholipids) do not circulate as independent molecules but are carried by specific apolipoproteins to form macromolecular complexes, called lipoproteins. Plasma lipoproteins keep their component lipids soluble during circulation and provide an efficient mechanism for transporting their lipid contents to (and from) the tissues. Four major groups of lipoproteins have been identified and they are important physiologically and in clinical diagnosis. They are chylomicrons, very low density lipoproteins (VLDL or pre β lipoprotein), LDL (β lipoprotein) and HDL (α lipoprotein). They differ in their relative composition of cholesterol, triacylglyceroles, phospholipids and apoproteins.

Chylomicrons, the largest and least dense lipoproteins, predominately transport triacylglycerols to adipose tissue and muscle, but also deliver the absorbed dietary and biliary
cholesterol to the liver. Once most of the triacylglycerols have been delivered to the adipose tissue and muscle, the remnants of the lipoprotein, including cholesterol, apo-E, and apo-B48 are then delivered to and taken up by the liver through interaction with the chylomicron remnant receptor.

VLDL is smaller and more dense than chylomicrons. VLDL contains triacylglycerols, some cholesterol and cholesteryl esters and the apoproteins; apo-B100, apo-CI, apo-CII, apo-CIII, and apoE. VLDL delivers triacylglycerols and cholesteryl esters from the liver and distribute them throughout the body. When VLDL moves into the circulating blood, it is converted first to intermediate density lipoproteins (IDL) and then into low density lipoproteins (LDL). Lipoprotein lipase serves to remove the majority of fatty acids from both the VLDL and IDL, thus increasing the density of the lipoproteins while maintaining cholesterol and cholesteryl ester concentrations. The removal of fatty acids and the loss of all apolipoproteins except apoB-100 and apo(a) results in the formation of LDL.

LDL is the primary blood carrier of cholesterol and delivers cholesterol to all tissues. LDL can be absorbed by the liver and other tissues via receptor mediated endocytosis. The LDL receptor recognizes the main apolipoprotein of LDL, apo-B100, resulting in the intake of LDL and subsequently enzymatic hydrolysis of cholesteryl ester. A positive correlation exists between the incidence of coronary atherosclerosis and the plasma concentration of LDL cholesterol.

High density lipoprotein is the smallest but most dense lipoproteins in the body. Nascent HDL or HDL contains several types of apolipoproteins including apo-AI, II & IV, apo-CI, II & III, apoD and apoE. HDL contains protein, phospholipids, cholesteryl esters, and cholesterol. HDL is produced as a protein-rich particle in the liver and intestine, and serves as a circulating source of Apo-CI & II and ApoE proteins. The HDL protein particle accumulates cholesteryl esters through the esterification of cholesterol by lecithin:cholesterol acyl-transferase (LCAT). LCAT is activated by apo-AI on HDL. HDL returns the liver where cholesterol is removed by reverse cholesterol transport, thus, serving as a scavenger of free cholesterol. Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl esters from HDL to VLDL, IDL, and LDL in exchange of triacylglycerol, relieving the inhibition of LCAT activity in HDL.

5. Hyperlipidemia and causes

Hyperlipidemia, most common form of dyslipidemia, refers to elevation of lipoproteins and/or lipids. HDL, LDL, and VLDL vary in their atherogenicities. High levels of cholesterol particularly LDL cholesterol together with low levels of HDL cholesterol increase the risks for developing atherosclerosis [39]. Hyperlipidemia itself usually causes no symptoms but can lead to the development of symptomatic vascular disease, including coronary artery disease and peripheral arterial disease. There are two different types of hyperlipidemia, primary and secondary hyperlipidemia:

1. Primary hyperlipidemia is generally due to genetic causes, such as a mutation in a receptor or binding protein. This type of hyperlipidemia is often linked to family history. For
instance, defects in the essential components of lipid transportation and metabolism inherited from family. Examples include familial defect in LDL receptor or apo B-100 (diminished LDL clearance and hypercholesterolemia), familial lipoprotein lipase deficiency (hypertriglyceridemia), and combination of multiple unknown defect and known familial defects (combined hyperlipidemia).

2. Secondary hyperlipidemia arises due to other underlying causes, such as sedentary lifestyle coupled with the excessive dietary intakes of saturated fat, cholesterol and trans-fats, in addition to many other disease conditions and drug uses. These factors include obesity, diabetes mellitus, hyperhomocystinemia, smoking, alcohol intake, chronic kidney disease, hypothyroidism, primary biliary cirrhosis and other cholestatic liver diseases, and drugs, such as thiazides, β-blockers, retinoids, estrogen and progesterons, and glucocorticoids.

6. Metabolic pathways involved in cholesterol homeostasis

6.1. Biosynthesis

Cholesterol levels in the body derived from de novo biosynthesis and diet. The majority of cholesterol utilized by healthy adults is synthesized in the liver, which accounts for about 70% of the daily cholesterol. Virtually all cells containing nucleus are capable of cholesterol synthesis, which occurs in endoplasmic reticulum and the cytosol. Biosynthesis of cholesterol generally takes place in the endoplasmic reticulum of hepatic cells and begins with acetyl-CoA, which is mainly derived from fatty acid oxidation reaction in the mitochondria. The conversion of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate by HMG-CoA reductase is the rate-limiting step of cholesterol biosynthesis and is under strict regulatory control. Thus, HMG-CoA reductase is one of important targets of cholesterol lowering drugs. The development of statin drugs is a very successful story of discovering and applying HMG-CoA reductase inhibitor to lower hypercholesterolemia.

6.2. Absorption

Dietary cholesterol is absorbed within the lumen of the small intestine. Bile salts produced from cholesterol in the liver interact with phospholipids to produce a biliary micelle that is transported via bile into the lumen. Dietary cholesterol in the lumen is easily incorporated into the micelles and together with the biliary cholesterol can be absorbed into the enterocytes. In the enterocytes, absorbed cholesterol is esterified by acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT2), which is found in both the intestine and liver. Reducing the absorption of cholesterol of dietary ad biliary sources has become another key area in cholesterol research and product development. A typical example is plant sterols/stanols that have long found as effective inhibitors of cholesterol absorption. These molecules inhibit cholesterol absorption by competitively inhibiting with cholesterol for incorporation into micelles. Recently, inhibitors, such as ezetimibe, that block the absorption of cholesterol into the enterocytes through...
suppressing the activity of cholesterol transporters have also been used to reduce absorption of dietary cholesterol.

6.3. Transport

Chylomicrons deliver absorbed dietary and biliary cholesterol from the enterocytes to the liver. During the process, triacylglycerols are released with assistance of lipoprotein lipase and taken up by adipose tissues and muscle, the remnants of the lipoprotein then delivered to, and taken up by, the liver through interaction with the chylomicron remnant receptor. In the liver, absorbed cholesterol, together with synthesized cholesterol and cholesterol transported back from peripheral tissues and LDL receptor-mediated uptake go through several metabolic pathways and secreted out from different outputs. One of them is the secretion in VLDL back into the bloodstream. VLDL removes triacylglycerols and cholesteryl esters from the liver and distributes them throughout the body. Endothelial Lipoprotein lipase remove the majority of fatty acids from both the VLDL and IDL, thus increasing the cholesterol and cholesteryl ester concentrations and apoB-100 results in LDL. LDL is the primary plasma carrier of cholesterol, which can be taken up by the liver and other tissues via receptor-mediated endocytosis. The cytoplasmic domain of LDL receptor facilitates the formation of coated pits which is the receptor-rich regions of the membrane. The ligand binding domain of the receptor recognizes apo-B100 on LDL, resulting in the formation of a clathrin-coated vesicle that buds from the inner surface of the cell membrane. ATP-dependent proton pumps lower the pH inside the vesicle resulting in dissociation of LDL from its receptor. After loss of the clathrin coat, the vesicles fuse with lysosomes, resulting in peptide and cholesteryl ester enzymatic hydrolysis. On the other hand, HDL is the small and rich in lipoproteins. The HDL protein particle accumulates cholesteryl esters by the esterification of cholesterol with lecithin:cholesterol acyl-transferase (LCAT). In the plasma, these particles undergo aseries of remodeling steps involving two HDL-associated proteins: phospholipid transfer protein (PLTP) and CETP. The primary role of PLTP is in the transfer of surface remnants, which contain apolipoproteins and phospholipids originating from triglyceride-rich lipoproteins, to pre-β-HDL. PLTP has also been implicated in mediating fusion of HDL particles to generate pre-β-HDL and CE-rich HDL. CETP promotes both transfer and exchange of hydrophobic lipids, CE, and triacylglycerols between lipoproteins. HDL can acquire cholesterol from cell membranes and transfer cholesteryl esters to VLDL and LDL via the transferase activity of apoD. More importantly, HDL can return to the liver where cholesterol is removed by reverse cholesterol transport, thus serving as a scavenger of free cholesterol. Scavenging activity of HDL initiating by accepting cholesterol from tissues in smaller HDL₃ via the ATP-binding Cassette transporter -1 (ABC-1). The cholesterol in HDL₃ is then esterified by LCAT, increasing the size of the particles to form the less dense HDL₂. The cycle is completed by the reformation of HDL₃ either after selective delivery of cholesteryl esters to the liver via the scavenger receptor-B1 or by the hydrolysis of HDL₂ phospholipid and triacylglycerol by hepatic lipase. HDL₂ concentration is inversely related to the incidence of coronary atherosclerosis. The enzyme cholesterol esterase controls
the hydrolysis of these stored cholesterol esters, yielding bioavailable cholesterol and fatty acids.

6.4. Excretion of cholesterol

About 1 g of cholesterol is eliminated from the body per day, approximately equivalent to the amount that absorbed cholesterol and synthesize cholesterol. Approximately, half is excreted in the feces after conversion to bile acids in liver, and the remainder is excreted as cholesterol. Bile acids serve to remove unwanted cholesterol from the body and to aid in lipid digestion in the intestine. 7α-hydroxylase, the rate limiting enzyme of bile acid biosynthesis converts cholesterol into 7-hydroxycholesterol. 7-hydroxycholesterol is converted to one of the two primary bile acids, cholic acid and chenodeoxycholic acid. Bile acids are then delivered to the intestines where they aid in the absorption of lipids. Some of bile acids are modified to form secondary bile acids (lithocholic acid and deoxycholic acid) in the intestine by intestinal bacteria. However, the majority of bile acids delivered to intestine are recycled by re-absorption in the ileum and returned to the liver by enterohepatic circulation. In liver, glyco- and tauroconjugate bile acids are formed and stored in gall bladder, from where they are released into the intestinal lumen for aid fat/lipids digestion and absorption.

7. Regulation of specific pathways and its influence on cholesterol homeostasis

Blood cholesterol concentration is a result of balance between cholesterol input and cholesterol output. When the input is surpass the output, blood cholesterol increases and by contrast, when cholesterol input is less than the output blood cholesterol levels decrease. Cholesterol input is attributed from the intestinal absorption of dietary and biliary cholesterol and cholesterol biosynthesis. On the other hand, the cholesterol output is mainly from LDL-receptor mediated LDL-cholesterol clearance, reverse transport by HDL, cholesterol catabolism by converting into bile acids, cholesterol and bile acids secretion in bile into the intestine lumen, and fecal excretion.

Metabolic nuclear receptors serve a central role in maintainingcellular and whole-body cholesterol homeostasis [40]. Two important transcriptional mechanisms to regulate cholesterol metabolism are the pathways mediated by sterol responsive element-binding protein (SREBP) and liver X receptor (LXR), which tightly regulate intracellular sterol concentrations. The SREBP pathway ensures that there is sufficient cholesterol to meet cellular requirements by directly activating expression of genesinvolved in the synthesis and uptake of cholesterol, and lipogenesis [41]. In the setting ofexcess free or unesterified cholesterol, SREBP-dependent gene expression is suppressed. LXR and farnesoid X receptor (FXR),together with other members of the nuclear receptor superfamily promote sterol storage, transport, and catabolism to prevent cholesterol accumulation [42]. LXRs respond to elevated cholesterol levels via transactivation of genes involved in sterol transport (ABCA1, ABCG1, ABCG5, and ABCG8), cholesterol efflux and high-density lipoprotein (HDL) metabolism (ABCA1, APOE, CETP, and
PLTP), and sterol catabolism (CYP7A1) [42]. Other members of metabolic nuclear receptor family include receptors for bile acids (CAR and PXR), and fatty acids (peroxisome proliferator-activated receptors). Through the coordinated regulation of gene transcription, these nuclear receptors regulate the key aspects of cellular and whole-body sterol homeostasis, including cholesterol absorption and synthesis, lipoprotein synthesis and remodeling, lipoprotein uptake by peripheral tissues, reverse cholesterol transport, and bile acid synthesis and absorption.

The amount of cholesterol that is synthesized in the liver is tightly regulated by dietary cholesterol levels. When dietary intake of cholesterol is high, synthesis is decreased and when dietary intake is low, synthesis is increased. However, cholesterol produced in other tissues is under no such feedback control. Cholesterol and similar oxysterols (the oxygenated derivatives of cholesterol, such as 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 27-hydroxycholesterol, and cholestenonic acid) act as regulatory molecules to maintain healthy levels of cholesterol. In tissues, many factor influence cholesterol balance through every cholesterol metabolic pathways.

7.1. LDL receptor-mediated cholesterol clearance

Cellular cholesterol increase is due to the uptake of cholesterol containing lipoproteins by receptors. LDL receptor regulates the cellular transport of LDL particles. One mechanism for regulating LDL receptor expression and controlling the expression of all the enzymes in the cholesterol biosynthetic pathway involves sterol-sensitive response elements (SREs). SREs are found in the promoters of the genes coding for the enzymes of cholesterol biosynthesis pathway and LDL receptors. Transcription factors of SRE activation are SREBP-3s. Three major SREBP isoforms, SREBP-1a, -1c, and -2, have been identified and differ in relative abundance in the liver and other various tissues. SREBP-1a is a potent activator of all SREBP-responsive genes and functions to maintain basal levels of cholesterol and fatty acid synthesis. SREBP-1c selectively activates genes involved in fatty acid synthesis, while SREBP-2 preferentially regulates genes important for cholesterol homeostasis by activating the transcription of HMG-CoA synthase, HMG-CoA reductase, LDL receptor [41].

Due to their ability to bind SREs, SREBP-2 plays an instrumental role in cholesterol homeostasis. These transcription-regulatory proteins are bound by another protein called SREBP cleavage activating proteins (SCAPs). SCAP, in turn, can bind reversibly with another endoplasmic reticulum-resident membrane protein, INSIG. SCAPs bind to SREBP-2 in the endoplasmic reticulum where a regulatory domain within SCAP responds to the level of oxysterols present in the cell. When the intracellular cholesterol and oxysterols concentrations decrease, the SREBP/SCAP complex moves to Golgi apparatus, leaving INSIG. Two proteases localized in Golgi, site-1 and -2 proteases (S1P and S2P) cleave SREBP-2 to release the transcription activation domain of SREBP-2. SREBP-2 preferentially activates transcription of target genes of LDL receptor [41]. When oxysterol levels are high, the SCAP/SREBP complex remains in the endoplasmic reticulum, preventing cleaved SREBP-2 from promoting gene expression. In addition to the up-regulation of LDLR transcription, nuclear SREBP-2 increases the transcription of PCSK9, a sterol-responsive protein that accelerates LDLR turnover in the
liver, thereby limiting lipoprotein uptake. As high concentrations of cellular cholesterol suppress SREBP-2 cleavage and release from endoplasmic reticulum, PCSK9 transcription is reduced, which subsequently increases LDLR levels, helping to maintain cholesterol homeostasis [43].

7.2. Regulation of cholesterol biosynthesis

SREBP-2 directly activate the expression of more than 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triacylglycerols, and phospholipids, as well as the reduced nicotinamide adenine dinucleotide phosphate (NADPH) cofactor required to synthesize these molecules. SREBP-2-responsive genes in the cholesterol biosynthetic pathway include those for the enzymes HMG-CoA synthase, HMG-CoA reductase, farnesyl diphosphate synthase, and squalene synthase. SREBP-1c and SREBP-2 activate three genes required to generate NADPH, which is consumed at multiple stages in these lipid biosynthetic pathways [41]. High cholesterol/oxysterol levels acting on SCAP ultimately stop the maturation of SREBP-2, resulting in the down regulation of key enzymes such as HMG-CoA reductase, thus, reducing the amount of cholesterol produced by the liver. To compensate the decreased cholesterol synthesis a homeostatic response in which cells increase the density of LDL receptors on their surfaces. This increases the clearance rate of LDL particles from the plasma and reduces plasma LDL cholesterol and its related health risks. The decrease in cholesterol synthesis also promotes an increase of HDL, thus, clearing even more cholesterol from the plasma.

Elevated levels of cellular cholesterol are accompanied by the increased production of oxysterols, which are specific ligands of LXR, allowing LXR to function as cholesterol sensors [44]. LXR respond to elevated cholesterol levels via transactivation of genes involved in sterol transport (ABCA1, ABCG1, ABCG5, and ABCG8), cholesterol efflux and HDL metabolism (ABCA1, APOE, CETP, and PLTP), and sterol catabolism (CYP7A1). Additionally, LXR also play a central role in regulating cellular lipid content through activation of SREBP-1c, which is the master regulator of de novo lipogenesis [40]. In response to activation, LXR act in a coordinated fashion to maintain cholesterol homeostasis by directing the tissue-specific expression of genes involved in sterol transport and metabolism [45]. A principal function of LXR in macrophages is to promote cholesterol removal from the cell through the induction of ABCA1, ABCG1, and apolipoprotein E. LXR also induces genes involved in lipoprotein metabolism, including LPL, CETP, and PLTP [45].

7.3. Regulation of cholesterol absorption and secretion

At the intestine, cholesterol is absorbed into enterocytes by a mechanism involving Niemann Pick C1-like protein 1 (NPC1L1) [46]. The NPC1L1 protein is abundant on intestinal brush border membranes. It functions as a sterol transporter to mediate intestinal cholesterol absorption and counterbalances hepatobiliary cholesterol excretion [46]. NPC1L1 is not under control of a nuclear receptor LXR [47]. In the enterocyte, cholesterol is readily esterified by the action of acyl-CoA:cholesterol acyltransferase 2 (ACAT2) and released into lymph in association with chylomicrons. The ATP-binding cassette (ABC) transporter protein ABCA1 and the ABC half-transporters, ABCG5 and ABCG8, are LXR target genes in the intestine and partici-
pate in cholesterol absorption. ABCA1 and ABCG5/8 counteract cholesterol absorption via efflux of cholesterol from the enterocyte into the gut lumen. LXR agonists exert their effect on cholesterol absorption through upregulation of ABCG5 and ABCG8, which is necessary for the majority of sterols secreted into bile [40, 48].

7.4. Regulation of cholesterol transport

The hepatic nuclear receptor, PPARα, exert control over many aspects of reverse cholesterol transport. Hepatic synthesis of apoA-I and apoA-II, the two major apolipoproteins in discoidal HDL (HDL₂), is regulated via PPARα activation and transcriptional regulation of RORα (NR1F1), a widely expressed nuclear receptor that is activated by cholesterol or cholesterol sulfate ligands [49]. ABCA1, which helps in reverse transport from peripheral tissues, is PPARγ/LXR-regulated cholesterol/phospholipid transporter [50]. PLTP is activated by both LXR and FXR whereas CETP is transactivated by LXR [40, 51].

7.5. Excretion of cholesterol

Bile acids are synthesized in hepatocytes and this production is tightly controlled by the nuclear receptor transcription factors, LXR-α and LXR-β. Activation of LXRs by specific oxysterol derivatives leads to the regulation of bile acid synthesis by stimulating cholesterol 7α-hydroxylase (CYP7A1) transcription to convert cholesterol to bile acids. FXR, a bile acid receptor, plays a central role of lipid metabolism in liver cells. FXR may play the major roles in bile acid metabolism, reverse cholesterol transport, and protect hepatocytes against cholestasis by feedback inhibition of bile acid synthesis by CYP7A1; stimulation of bile acid efflux from hepatocytes by bile salt export pump; inhibition of bile acid uptake into hepatocytes by Na⁺-taurocholate co-transporting polypeptide; and regulation of reverse cholesterol transport by inducing ApoCII and PLTP [40, 52].

8. Cholesterol-lowering drugs

Mainly cholesterol lowering drugs have been developed and used clinically, which includes 1) HMG-CoA reductase inhibitors, e.g., atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin, 2) bile acid sequestrants — colesevelam, cholestyramine and colestipol — and nicotinic acid (niacin), and 3) cholesterol absorption inhibitor - ezetimibe. Other available drugs are gemfibrozil, fenofibrate and clofibrate which are fibric acid derivatives primarily used for lowering high triglyceride levels

8.1. Statins – Benefits versus side effects

Statins or inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase are widely used to reduce the risk of cardiovascular events and death. Statins includes lovastatin, which is a fungal metabolite, and many synthetic derivatives, pravastatin, atorvastatin and simvastatin etc. Statins target predominantly hepatocytes and inhibit HMG-CoA reductase,
the enzyme that converts HMG-CoA into mevalonic acid, a cholesterol precursor. Statins alter the conformation of the enzyme when they bind to its active site. This prevents HMG-CoA reductase from attaining a functional structure. The change in conformation at the active site makes these drugs very effective and specific [53]. Binding of statins to HMG-CoA reductase is reversible, and their affinity for the enzyme is in the nanomolar range, as compared to the natural substrate, which has micromolar affinity [54]. The inhibition of HMG-CoA reductase determines the reduction of intracellular cholesterol, inducing the activation of a protease which slices SREBPs from the endoplasmic reticulum. SREBPs are translocated into nucleus, where they increase the gene expression for LDL receptor. The reduction of cholesterol in hepatocytes leads to the increase of hepatic LDL receptors that leads to the reduction of circulating LDL and of its precursors (intermediate density - IDL and very low density- VLDL lipoproteins). All statins reduce LDL cholesterol non-linearly, dose-dependent, and after administration of a single daily dose [53]. Statins inhibit hepatic synthesis of apolipoprotein B-100, determining a reduction of the synthesis, secretion of triacylglycerols-rich lipoproteins, and an increase of receptors for apolipoproteins B/E. Statins have a modest effect on HDL increase, and no influence on lipoprotein(s) concentration. Statins can also prevent LDL oxidation by preserving the activity of the endogenous antioxidant system, like superoxide dismutase [53].

In general, the currently used statins are well tolerated and have a good safety profile [55]. Statins acting as inhibitor of HMG-CoA reductase, inhibits all the pathway in which mevalonate is a precursor such as synthesis of nonsteroid isoprenoids such as farnesylpyrophosphate (FPP), geranylgeranylpyrophosphate (GGPP), dolichol and side chain of coenzyme Q which play an essential role in cellular physiology. Coenzyme Q is a component of mitochondrial respiratory chain and a lipid-soluble antioxidant. Farnesyl- and geranylgeranyl groups are needed for protein isoprenylation and formation of small GTP-binding proteins including Ras, Rho and Rab, which are involved in signal transduction pathways. Inhibition of isoprenoid synthesis by statins decreases the activity of these proteins and modifies the respective signalling pathways; the mechanism responsible for cholesterol-independent pleiotropic effects of statins. The best recognized and most commonly reported adverse effect of statins are muscle adverse effect with muscle pain, fatigue and weakness as well as rhabdomyolysis. Rhabdomyolysis is the most severe form of statin induced myopathy, characterized by marked increase in CK activity, myoglobinemia, myoglobinuria, and myoglobin induced acute renal failure. These symptoms arising on statins are shown to be reversed with discontinuation. Coenzyme Q10 deficiency produces mitochondrial encephalomyopathy, resulting in fatigue, muscle symptoms, and cognitive problems. Gastrointestinal and neurological symptoms, psychiatric symptoms, sleep problems, glucose elevations, and a range of other symptoms are also reported on statins [56].

8.2. Ezetimibe – Benefits and side effects

Ezetimibe is the first of a new class of highly selective cholesterol absorption inhibitors. It does not inhibit cholesterol synthesis in the liver or increase bile acid excretion. It belongs to a class of lipid-lowering compounds that selectively inhibits the intestinal absorption of cholesterol
and sterols. Ezetimibe’s pharmacological effect is complementary to that of the statins [57]. Mechanism of action of ezetimibe involves inhibiting the absorption of cholesterol in the small intestine. Through a mechanism that is not yet fully elucidated, ezetimibe appears to block a protein transporter called Niemann-Pick C1-like 1 protein (NPC1L1) that is located at the apical membrane of the small intestine enterocytes [58]. Unlike other cholesterol-lowering agents, ezetimibe localizes and appears to act at the brush border of the small intestine and inhibits the absorption of dietary and biliary cholesterol in the small intestine, leading to a decrease in the delivery of intestinal cholesterol to the liver. This leads to a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood. Ezetimibe has been demonstrated to have no significant effect on the plasma concentrations of the fat-soluble vitamins A, D, and E [57]. No published reports could be identified that assessed the potential impact of ezetimibe therapy on other traditional CVD risk factors, including blood pressure and obesity. Recent reports have raised concerns about an association between ezetimibe and an increased incidence of cancer [58]. Ezetimibe may rarely cause hepatotoxicity, severe cholestatic hepatitis, or acute autoimmune hepatitis [59].

Ezetimibe is proved to be more effective in combination with statin drugs in lowering LDL-c than monotherapy. Ezetimibe has an additive and at times synergistic effect on the reduction of LDL-C and total cholesterol (TC) concentrations when combined with statin therapy. Ezetimibe has not been associated with increased rates of myopathy or rhabdomyolysis, whether used alone or in combination with statins, although there have been some case reports of myopathy attributed to this agent. Moreover, ezetimibe has been associated with mild elevations of liver transaminases, mainly in combination with a statin. Other side effects are extremely rare. It should be noted, however, there are no long-term safety data or outcome studies for ezetimibe yet [60].

8.3. Niacin (Nicotinic acid)

Niacin is the most potent HDL increasing drug currently available and it also can efficaciously lowers triglycerides and LDL cholesterol. It is the only lipid-lowering drug that considerably lowers lipoprotein(a). Beneficial effect of niacin to reduce triglycerides and apolipoprotein-B containing lipoproteins (e.g., VLDL and LDL) are mainly through: a) decreasing fatty acid mobilization from adipose tissue triglyceride stores, and b) inhibiting hepatocyte diacylglycerol acyltransferase and triglyceride synthesis leading to increased intracellular apo B degradation and subsequent decreased secretion of VLDL and LDL particles. Niacin raises HDL by decreasing the fractional catabolic rate of HDL-apoAI without affecting the synthetic rates. Additionally, niacin selectively increases the plasma levels of lipoprotein-AI (HDL subtraction without apoAII), a cardioprotective subtraction of HDL in patients with low HDL. Recent studies indicate that niacin selectively inhibits the uptake/removal of HDL-apoAI (but not HDL-cholesterol ester) by hepatocytes, thereby increasing the capacity of retained HDL-apoAI to augment cholesterol efflux through reverse cholesterol transport pathway [61]. Niacin treatment is associated with a number of side effects, including headache, itching and gastrointestinal disturbances, but these are generally mild. The most severe of the side effects is flushing, and this is sufficiently severe to negatively affect compliance [62].
8.4. Bile acid sequestrant

Bile acid sequestrant or bile binding anion (Chloride) exchange resins that is effective in reducing total cholesterol and LDL cholesterol levels. The primary and direct action of the bile acid sequestrants is to bind to bile acids in the gut and thus interrupt the enterohepatic recirculation of bile acids [63]. Three key enzyme are affected by bile acid sequestrant, which are phosphatidic acid phosphatase, cholesterol 7-alpha-hydroxylase and HMG-CoA reductase. Activation of phosphatidic acid phosphatase promotes hepatic triglyceride synthesis, induces secretion of triglyceride-rich VLDL particles and consequently increases plasma triglyceride levels. The activation of hepatic cholesterol 7-alpha-hydroxylase promotes the conversion of intracellular cholesterol to bile acids. The decrease in intracellular cholesterol stores, in turn, increases LDL receptor expression on hepatocyte membranes and consequently, increases receptor-mediated fractional catabolism of LDL or LDL uptake by liver cells. Reduction of intracellular cholesterol may also increase the synthesis of cholesterol through activation of HMG-CoA reductase. The potential loss of the bile acid sequestrant’s cholesterol-lowering efficacy can be overcome by adding HMG-CoA reductase inhibitor (statins). Finally, bile acid sequestrants promote apoprotein AI synthesis and tend to raise high-HDL cholesterol. Three drugs in this class are synthetic cholestyramine, colestipol, colesevelam. The side effect profile of the bile acid sequestrants is tolerable, with most complaints related to effects on the gastrointestinal tract and the bulkiness of resins [64].

8.5. Fibrates

Fibrates are primarily effective for the treatment of hypertriglyceridemia or mixed hyperlipidemia by stimulating the peroxisomal β-oxidation pathway. Their main action is to lower plasma triglyceride levels, but they also reduce total and LDL cholesterol concentrations and induce a moderate increase in HDL cholesterol. Fibrates act by stimulating the activity of peroxisome proliferator-activated receptor (PPAR)-α, a member of the PPAR subfamily of nuclear receptors [65]. It controls the transcription of regulatory genes of fatty acids and cholesterol metabolism. It inhibits the synthesis and secretion of triglycerides by the liver and a stimulation of the degradation of triglyceride-rich lipoproteins. This increased clearance of triglycerides results from a stimulation of the expression of lipoprotein lipase and a decreased expression and concentration of apolipoprotein-CIII, an inhibitor of lipoprotein lipase activity. PPAR-α activation modifies the expression of several key genes controlling HDL cholesterol metabolism and reverse transport of cholesterol [65]. Several fibrate drugs such as ciprofibrate, bezafibrate, fenofibrate, and gemfibrozil has revolutionized lipid-lowering but research has shown the prolonged use of some of these drugs like clofibrate and ciprofibrate causes peroxisome proliferation leading to hepatomegaly and tumor formation in the liver of rodents [66].

A recent report demonstrated that between the periods 1988-1994 and 1999-2002, mean total cholesterol and mean LDL cholesterol declined in American adults. Coincidently, during this time there also was an increase in the percentage of adults receiving lipid-lowering medications. However, among adults not receiving lipid-lowering medications, trends in lipids were
similar to those reported for adults overall. Among obese adults, mean total cholesterol, non-HDL cholesterol, LDL cholesterol, and geometric mean triglycerides declined between 1988 and 2010[67]. These data suggest that in addition to the increased use of lipid-lowering medications, something else must have been involved in the overall reduction of blood total cholesterol and LDL cholesterol. Although those factors, the application of natural lipid-lowering products plays a critical role and is thus described below.

9. Cholesterol-lowering natural products

9.1. Plant sterols/ stanols

Plant sterols/ stanols, naturally occurring in foods of plant origin, perform similar biological functions to cholesterol, and contain a similar chemical structure. They differ from cholesterol only in the presence of either an extra methyl or ethyl group. Absorption efficiency for plant sterols in humans is less (2-5%) than that of cholesterol (60%). The most common forms are unsaturated plant sterols β-sitosterol, campesterol, and stigmasterol and the saturated sitostanol and campestanol [68]. Plant sterols reduce the absorption of both dietary and biliary cholesterol from the intestinal tract by 30%-50% [69]. The exact mechanism is yet fully elucidated while it is generally assumed that the presence of increased quantities of plant sterols in the gut lowers the micellar solubility of cholesterol, therefore lowering the amount of cholesterol available for absorption [69]. Intake of phytosterol and stanols at an average of 2 g/day has been shown to lower low density lipoprotein cholesterol (LDL-C) by 10-15%. The effect appeared to be peaked at intakes of 2 g/d, with little additional benefit being achieved at intakes higher than 2.5 g/d. The recommended daily intake of phytosterols is 2 g/d in humans [70]. Plant sterols and their derivatives reduce plasma cholesterol levels independently from the mRNA expression of ABCG5 and ABCG8 transporters [71]. Food products such as margarine, milk, yoghurt, and cereal products enriched with plant sterols/stanols are promoted as functional foods to help lower serum cholesterol levels. Human and animal studies have shown that plant sterol and stanol esters are non-toxic [72]. There have been concerns raised over the reduced absorption of some fat soluble vitamins from the use of plant sterols. For example, plant sterols and stanols have been shown to reduce β-carotene, α-carotene, and vitamin E levels by around 25%, 10%, and 8%, respectively [72]. Used alone in the diet, or as an adjuvant to drug therapy, or in combination with other functional food components, plant sterols/stanols-enriched products are generally effective at reducing serum total and LDL-C [72], and thus the most popular natural ingredient in cholesterol-lowering product market.

9.2. Soy products

Soy foods have been consumed for centuries in Asian countries. Consumption of soy foods contribute to lower incidences of coronary heart diseases, atherosclerosis, type 2 diabetes, and decreased risk of certain types of carcinogenesis such as breast and prostate cancers [73]. Animal and human studies have also shown that consumption of soy protein or associated isoflavones has beneficial impacts such as lowering liver or blood triglyceride, total and LDL
cholesterol levels, increasing HDL cholesterol and the ratio of HDL/LDL cholesterol [73, 74]. Soy protein regulates SREBP-1 expression by modulating serum insulin concentration, thus preventing the development of fatty liver [75]. Isoflavones are major soy phytoestrogens present in soy foods and Genistin, daidzin, and glycitein are the main soy isoflavones (Xiao 2008). Soy and soy bioactive components are well-tolerated and the adverse effects reported are gastrointestinal symptoms (e.g., diarrhea), followed by menstrual complaints (e.g., prolonged periods, amenorrhea) headache, dizziness, and musculoskeletal complaints [76].

9.3. Dietary fibre

Dietary fibre is one of the most studied dietary components associated with cardiovascular benefits. It is a complex of non-digestible carbohydrates and lignin that are intrinsic and intact in plants and are resistant to digestion and absorption in the small intestine. Dietary fibre can modulate body weight and promotes beneficial physiological effects such as laxation, reduction in blood cholesterol and postprandial blood glucose [77]. Traditionally, dietary fibre has been classified on the basis of its solubility in water (soluble or insoluble). Foods rich in fibre need to be chewed longer, leading to an increase in the time needed to eat and the feeling of satiety. Fibres which make up viscous solutions also delay the passage of food from the stomach to duodenum and contribute to an increase in satiety and a decrease in energy consumption [78]. In the intestine, the incorporation of fibre in food may complicate the interaction between digestive enzymes and their substrates, thus slowing down the absorption of nutrients[77]. The hypocholesterolemic action of fibre is partly mediated by a lower absorption of intestinal bile acid because the interruption of the enterohepatic bile acid circulation, thus increasing faecal bile acid loss, and its de novo synthesis in liver. The physicochemical properties of soluble fibre result in important modifications in volume, bulk and viscosity in the intestinal lumen, which will alter metabolic pathways of hepatic cholesterol and lipoprotein metabolism, also resulting in lowering of plasma LDL cholesterol [79]. Dietary fibre increases the enzymatic activity of cholesterol-7-α-hydroxylase, contributing to a higher depletion of hepatic cholesterol but increased endogenous cholesterol synthesis. However, there is an increase in the number of LDL receptors and in the recruitment of the esterified cholesterol from the circulating LDL particles. Several types of soluble dietary fibre such as pectin, glucomannan, psyllium can decrease plasma total cholesterol and LDL cholesterol. Epidemiological evidences showed a stronger association of cardiovascular protection with soluble fibre than insoluble fibre. Insoluble fibre such as that from wheat or cellulose has not been reported to have any significant effect on blood cholesterol [78]. Consumption of too much high fibre foods that have not been cooked can cause side effects of abdominal bloating and gas.

9.4. Flaxseed lignans

Flax seed is the richest source of natural lignans, with secoisolariciresinol diglucoside (SDG) being the principal lignan compound. Flaxseed or flaxseed meal have cardioprotective properties and can suppress atherosclerosis by virtue of its antioxidant properties due to the presence of flaxseed lignans. Lignan reduces serum triglycerides and LDL and raises HDL
cholesterol. In addition, flaxseed oil possesses anti-inflammatory properties and reduces platelet aggregation as well [80]. Flaxseed lignans along with soy isoflavones are phytoestrogens commonly consumed in the human diet.

9.5. Polyunsaturated fatty acids and omega-3 fatty acids

An old assumption regarding fatty acids and their effects on atherosclerosis was that monounsaturated fatty acids were neutral, saturated fatty acids were bad, and polyunsaturated fatty acids were good. However, a study conducted by Scott Grundy and Fred Mattson in 1985 turned the “world of monounsaturated fatty acids” around[81]. They demonstrated that diets rich in saturated fatty acids caused a high LDL cholesterol/HDL cholesterol ratio, and that substitution of monounsaturated fatty acid for saturated fatty acids reduced LDL cholesterol but did not reduce HDL cholesterol. Consequently the LDL cholesterol/HDL cholesterol ratio was the lowest with monounsaturated fatty acids, given that polyunsaturated fatty acids reduced HDL cholesterol as well as LDL cholesterol. A similar phenomenon was observed in a 5-year study in male African green monkeys[82]. In the monkey studies, average HDL cholesterol was 50 mg/dl in the polyunsaturated fatty acids group versus 86 and 81 mg/dl in the saturated fatty acids and monounsaturated fatty acids groups, respectively. Average plasma LDL cholesterol concentrations in the polyunsaturated fatty acids and monounsaturated fatty acids-fed monkeys were 157 and 167 mg/dl, respectively (no significant difference between them) versus 257 mg/dl in the saturated fatty acids-fed animals.

The influences of dietary fatty acids on blood cholesterol profiles are also related to diet composition[83]. It is well accepted that the consumption of saturated fatty acids increases LDL cholesterol, whereas carbohydrates, monounsaturated fatty acids and polyunsaturated fatty acids do not. The effect of fatty acids on blood lipid profiles also depends on heath conditions. Among individuals who are insulin resistant, a low-fat, high-carbohydrate diet typically has an adverse effect on lipid profiles. In addition to lowering HDL cholesterol, it also increases triacylglycerols and LDL cholesterol. Consequently, a moderate fat diet in which unsaturated fatty acids replace saturated fatty acids and carbohydrates are not augmented is advised to lower LDL cholesterol[83].

Fish oil (marine n-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid), whether from dietary sources or fish oil supplements, exhibit cardioprotective effects and reduce mortality due to cardiovascular diseases. Fish oil provides cell membrane stabilization, anti-inflammatory, antiatherogenic effects and suppression of cardiac arrhythmias[84]. Omega-3 fatty acids lower moderately the blood pressure through primarily the improvement of vascular endothelial cell function whilst a multitude of mechanisms may be involved85. Polyunsaturated fatty acids lower triacylglycerols, which has also been important in cardioprotection and the management of insulin resistance and diabetes. The effect of omega-3 fatty acids on blood cholesterol is inconsistent effect. In general, omega-3 fatty acids do not offer a benefit of directly lowering blood cholesterol. Instead, in some studies fish oil has been found to cause a small rise in LDL-cholesterol; however a change in the LDL particle size from the smaller more atherogenic form to the larger less damaging particle size have been noted85.
Health Canada has recently reconsidered the classification of food products with disease risk reduction claims or therapeutic claims in light of clarified principles for the classification of foods at the Food-Natural Health Product interface (http://www.hc-sc.gc.ca/fn-an/label-etiquet/claims-reclam/assess-evalu/sat-mono-poly-fat-gras-eng.php). Health Canada has concluded that the results of the updated literature review are consistent with the 2002 report provided by the Institute of Medicine (IOM), which forms the basis of the US and Canada dietary guidance, on the replacement of saturated fat with unsaturated fat for blood cholesterol lowering. In other words, scientific evidence exists in support of the therapeutic claim linking the replacement of saturated fat with unsaturated fat to a reduction of blood cholesterol. It is stated that the claim is relevant and generally applicable to the Canadian population as a high proportion of the Canadian population is hyperlipidemic. It is allowed now by the Health Canada’s to put therapeutic claim statements such as “Replacing saturated fats with polyunsaturated and monounsaturated fats (from vegetable oils) helps lower/reduce cholesterol” in vegetable oils and foods containing vegetable oils when specific conditions for the food carrying the claim are met.

9.6. Olive oil

Olive oil can reduce LDL and raise high-density lipoprotein cholesterol and decrease lipid damage due to oxidative stress. In addition, olive oil reduces inflammatory and thrombogenic status, endothelial dysfunction, and blood pressure[86].

9.7. Green tea products

Tea catechin—especially (−)-epigallocatechin-3-gallate—inhibits the expression of soluble adhesion molecules including vascular adhesion molecule-1 and intercellular adhesion molecule-1, endothelial cell inflammatory markers, decreased oxidized LDL, and prevents the development of atherosclerosis and[87].

Certainly, there are more natural products available, for instance a number of antioxidants and phenolic compounds, to lower blood cholesterol levels. However, the big challenges that the natural products have been facing in the past years lie in their relatively lower efficacies as compared with the cholesterol-lowering drugs. The apparent advantages of natural products are their better safety profiles. In order to promote the market share of cholesterol-lowering natural products in competing with the drugs, it is critical to develop new products that can offer better efficacies than the current natural products, without losing safety or introducing increased toxic or severe side effects. Considering that the majority of the current natural products fall into the same category of cholesterol absorption inhibitor, the future direction of research and development of cholesterol-lowering products, novel distinct pathways or targets should be focused. Herewith, we will provide some brief thoughts on new approaches that we believe worth to tackle into, with a hope that the new mind in the research and development direction and focus would help to discover and develop novel natural products with significantly improved cholesterol-lowering efficacy working through distinct mechanisms than the currently available natural products, without apparent side effects.
10. New perspectives on the development of natural cholesterol-lowering products

Cardiovascular friendly natural products are those nutritional supplements, which can provide potential health benefits in cardiovascular diseases (CVD). Experimental, epidemiological and clinical data indicates that dietary nutrients and supplements have profound cardioprotective effects in the primary as well as secondary prevention of coronary heart disease [88]. Except for the aforementioned several cholesterol-lowering natural products, the plant alkaloid berberine has recently been introduced to the field of lipid-lowering products. Berberine has shown a moderate or comparable cholesterol- and triacylglycerol-lowering effect in humans [89]. More importantly, recent studies have demonstrated that berberine combined with plant stanols improves cholesterol-lowering efficacy through a synergistic action on cholesterol absorption and reducing plasma triacylglycerols in animals [90], [91], with the efficacies are comparable or even better cholesterol-lowering drugs, statins. The product has been patent-protected and at the preclinical stage. Clinical trials are warranted before moving this potentially highly effective natural product to the lipid-lowering market.

10.1. Current status in the development of cholesterol-lowering products

An initial approach to control the modifiable risk factors of hyperlipidemia relies on the changes of diet, lifestyle, and other factors such as smoking. When all these fail, pharmacological intervention is necessary. Many of the current available drugs are those either inhibit cholesterol synthesis, or increase synthesis of bile acids and excretion. The majority of the current research focuses on lowering the “bad” LDL cholesterol, with less attention on increasing “good” HDL cholesterol. A treatment with a combination that can lower LDL cholesterol and increase HDL cholesterol would certainly provide more benefits than single approach for the treatment of hyperlipidemia, atherosclerosis and cardiovascular disease.

10.1.1. Lowering LDL cholesterol

Statins drugs, which are currently widely prescribed, lower LDL cholesterol primarily via inhibiting cholesterol biosynthesis and a secondary effect of increase LDL receptor and LDL clearance. However this family of drugs has shown several undesirable side-effects. Niacin, can also lower LDL cholesterol but not efficiently as statin but it is only one drug proved to increase HDL cholesterol with very less side effects. Therefore, a combination of niacin and statins might be superior to these drugs alone for lowering lipid levels. The scientific evidence of long-term safety and tolerability of these combination therapies are key determinants for good compliance and cardiovascular benefits.

Proprotein convertase subtilisin kexin-9 (PCSK9) is a sterol-responsive protein that accelerates LDLR turnover in the liver. When PCSK9 transcription is reduced, there will be subsequently increases LDLR levels helping to maintain cholesterol homeostasis. The transcription of PCSK9 is increased by SREBP-2, which also regulate the transcription of many other genes. Therefore,
a pharmacologically specific inhibitor of PCSK9 will have atherogenic effect by increasing LDLR levels without affecting other genes regulated by SREBP-2.

### 10.1.2. Increasing HDL levels

Low HDL-cholesterol (HDL-C) is a strong and independent cardiovascular risk marker. Niacin is the only drug proved to increase HDLC but associated with flushing as a side-effect. Niacin-induced flushing involves both PGD\textsubscript{2} from mast cells and serotonin from platelets. The possibility of administering or formulating niacin together with flavonoid luteolin was recently shown to inhibit niacin flush in rats. Further investigation on inhibitors on side-effect can warrant an effective medicine for CVD[92]. Aleglitazar, a dual PPAR\textsubscript{a}/\gamma agonist, proven has beneficial effects on both lipid and glucose parameters [93]. Activation of PPAR\textsubscript{a} may increase ApoA1, apolipoprotein of HDL and increased the synthesis of LPL which hydrolyzes VLDL, IDL and LDL. PPAR\textsubscript{a} agonist, with good safety profile, may have a therapeutic role in modifying cardiovascular risk factors.

### 10.1.3. CETP inhibition

Besides established strategies to increase HDL-C, e.g. with nicotinic acid, CETP (Cholesteryl ester transfer protein)-inhibition is a promising new therapeutic option. The failure of torcetrapib, the first CETP-inhibitor, seems to be attributed to "off-target" effects. Treatment with the newer CETP-inhibitors dalcetrapib and anacetrapib has been shown to be efficacious and safe - but their usefulness in clinical practice remains to be determined in ongoing clinical trials [94].

### 10.1.4. Inhibition of cannabinoid receptors

G protein-coupled cannabinoid receptor, CB-1 of the endocannabinoid system, plays a crucial role in regulating feeding pattern, lipid metabolism, and energy homeostasis. CB-1 receptors are located in the central nervous system and peripheral tissues including adipocytes, pancreas, gut, liver, and muscle. Rimonabant, a selective CB-1 antagonist drug to be developed for weight loss via increasing energy expenditure actually also increases HDL-cholesterol and triglycerides. However, the clinical efficacy and safety of these new antiobesity compounds are yet to be determined. Additional cannabinoid receptor blockers have been developed and are in testing [95].

### 10.1.5. Nuclear receptor modifiers

Identification of nuclear receptor LXR and FXR as regulator of genes in bile acid and cholesterol metabolism has provided potential new targets for screening cholesterol-lowering drugs by manipulating bile acid synthesis, transport, and absorption. Therapies targeted to LXR and FXR would be ideal for drug development because nuclear receptors are activated by natural and synthetic ligands, which could be identified by high-throughput screening [96].
10.1.6. Acyl-coenzyme A: Cholesterol acyltransferas (ACAT) inhibitors

ACAT1 and ACAT2 play an important role in cellular cholesterol esterification and thus modulate intestinal cholesterol absorption and hepatic lipoprotein secretion. ACAT2 is proposed to play a central role in cholesterol absorption from the intestine. ACAT1, on the other hand is widely distributed in tissues, and plays a pivotal role in cholesterol metabolism in macrophages and steroidogenic tissues. It is anticipated that inhibitors of ACAT2 activity could be developed that would decrease cholesterol absorption, whereas specific ACAT1 inhibitors could be used to reduce foam cell formation and prevent atherosclerosis. However, the results of studies have indicated that ACAT1 inhibition is not a good strategy and, in fact, could have detrimental consequences [97]. In contrast, mice lacking ACAT2 exhibited attractive metabolic findings. These include a restricted capacity to absorb cholesterol and protection against diet-induced hypercholesterolemia and gallstone formation [98, 99]. Further, ACAT2 inhibitor reduces cholesterol esters in apolipoprotein B-containing lipoproteins and protects from atherosclerosis in murine models of the disease [100]. A fundamental question remains: would ACAT2 specific inhibition in humans, either pharmacologically or with anti-sense oligonucleotides, prevent or reduce atherosclerosis? “The sole test of the validity of an idea is experiment.” Until a potent and specific inhibitor of ACAT2 is tested in humans, the hypothesis remains uncertain [101].

10.2. Future perspectives of cholesterol-lowering products and their potential impact on CVD

10.2.1. Identification of new molecular targets

Target identification is an essential first step in drug development. Although cholesterol metabolism has been studied for decades and relatively well understood, the specific molecular targets that can be used for drug discovery and development are far from well-known. It is believed that with the development and use of modern molecular technology and techniques in nutritional and physiological research, more targets will be identified and used for future drug development.

10.2.2. Application of gene silencing technology

Apart from the identification of new targets, several new approaches have been introduced or shown great potential for the development of new cholesterol-lowering products. Of them is gene-silencing technology. One technology that has generated a lot of excitement is RNA interference (RNAi) [102]. For example, clinical trials have been launched using RNA interference approaches to reduce PCSK9 expression or specific antibodies targeting and inhibiting PCSK9 interaction with the LDL receptor. They constitute very promising approaches to reducing cholesterol levels and coronary heart disease. Understanding of PCSK9 and its potential as a therapeutic target through which to reduce LDL cholesterol for prevention and treatment of coronary heart disease has been of great interest recently [103]. The administration of chemically modified small interference RNAs (siRNAs) results in silencing of a target mRNA, such as apolipoprotein B (apoB) mRNA in liver and jejunum and decreasing plasma...
levels of apoB protein. ApoB is a molecule involved in the metabolism of cholesterol; the concentrations of this protein in human blood samples correlate with those of cholesterol. Higher levels of both compounds are associated with an increased risk of coronary heart disease. Intravenous injections of the siRNA-cholesterol conjugates in mice resulted in a lowering of the levels of blood cholesterol comparable to that in mice in which the apoB gene had been deleted. These results demonstrate that siRNA can be delivered systemically to target the liver and suggest that RNAi has the potential to become a new therapeutic for the treatment of metabolic diseases [102].

10.2.3. Development of target-specific inhibitors or enhancers

For those molecular targets that have several different isoforms, the biggest challenge in the drug development is the specificity. A good example is ACAT inhibitors. To date, it has been demonstrated that there are two isoforms, ACAT1 and ACAT2. ACAT1 is expressed universally while ACAT2 is expressed mainly in the liver and small intestine. Both isoforms can esterify cholesterol and other sterols. However, ACAT1 primarily works on the esterification of plant sterols and other sterol products, while ACAT2 mainly converts cholesterol to its esters. Due to their different distribution and activities in the body, a universal inhibitor of both enzymes has been shown to be detrimental and recent humans trials are disappointing [104, 105]. The study of Bell et al. in 2006 breathes life back into the idea of ACAT2-specific inhibition [106]. In atherosclerosis-prone mice, ACAT2 was specifically inhibited in the liver with antisense oligonucleotides. Biweekly intraperitoneal injections, which reduced ACAT2 expression by a remarkable 80%, decreased diet-induced hypercholesterolemia and sharply reduced cholesterol ester deposition in the aorta. The specific inhibition can be achieved from the antisense gene technology or small molecules that inhibit only ACAT2. There is no mature product in this category and lots more work needs to be done.

It has been demonstrated by numerous studies that inhibition of cholesterol absorption is associated with much less side-effects than other approaches. It remains challenge but highly promising that a potent inhibitor of cholesterol absorption would sufficiently lower blood cholesterol or keep blood cholesterol levels within the recommended healthy range. To achieve this goal, other approaches have to be take in addition to the specific inhibitor of cholesterol absorption, such as a combination of two or more products, in particular the combinations that work synergistically through one or multiple pathways of cholesterol homeostasis. Recent studies on the combination of the plant alkaloid berberine and plant sterols/stanols have shown an excellent example of this approach. The researchers have demonstrated consistently that plant sterols or stanols and berberine have a moderate effect to lower blood cholesterol levels in different animal models. However, when plant sterols/stanols were combined with berberine, a significant synergism was produced. They remarkably improved cholesterol-lowering efficacy by synergistically inhibiting cholesterol absorption [90,91].
11. Conclusions

Cardiovascular disease has long been the lead cause of mortality and morbidity in the developed countries and similar situation has arisen in recent years in the developing countries. The increased prevalence of CVD is due to the significantly improved food supply and processing and rapid shift of lifestyle from active to sedentary nature, resulting increased incidence of obesity and overweight. Although the development and clinical use of statin drugs and other cholesterol-lowering drugs has been stabilize the mortality resulted from CVD since last decade, the incidence has been stably rising. According, the control of risk factors for atherosclerosis and CVD remains critical. The primary risk factor is hypercholesterolemia. There still exists a strong demand to develop novel products, especially from distinct cholesterol metabolic pathways or molecular targets and from different new approaches as mentioned above. It is optimistic that in the near future, new cholesterol-lowering products, either drugs or natural products with higher efficacies, better specificity and better safe profiles will be developed.

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