Molecular regulation of cholesterol metabolism: HDL-based intervention through drugs and diet

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The overloading of cholesterol in the arteries remains the principal cause of cardiovascular diseases. Since available anti-cholesterolemic drugs are not completely effective and have several severe adverse effects, the aim of this review is to analyze current research focused on the emerging, innovative therapeutic strategies based on both pharmacological and nutritional interventions to control cholesterol metabolism. Pharmacological interventions mainly involve the use of molecules capable of interfering with high-density lipoprotein (HDL) metabolism and the reverse cholesterol transport (RCT) through genetic control of apolipoprotein A-I (ApoA-I), agonism at liver X-receptor α (LXRα), or inhibition of cholesteryl ester transport protein (CETP), scavenger receptor BI(SR-BI), and ecto F1F0ATPase/synthase. Nutritional interventions are based on the use of fibres, phytosterols, and probiotics acting through interference with absorption and re-absorption of cholesterol by enterocyte and hepatocyte specific transporters, thus influencing RCT final step. The search for new drugs is still at the very beginning and new molecules are not yet ready to enter clinical use. However, several promising findings coming from innovative biotechnological research are expected shortly to produce probiotics, fibres, and phytosterols to be used as therapeutic tools. Among the most important advantages of natural products in respect to traditional drugs are the lack of severe adverse effects and their low cost.

Keywords: Cardiovascular disease, Cholesterol, Drugs interfering with RCT, HDL, Nutrition

Introduction

An elevated serum cholesterol level is still considered to be one of the major risk factors associated with atherosclerosis and cardiovascular diseases and the major cause of morbidity and mortality around the world. The World Health Organization (WHO) predicts that by the year 2020, up to 40% of all deaths will be related to cardiovascular diseases. The problem of cholesterol control is far away to be solved, since available antilipidemic drugs are not completely effective and have several severe adverse effects.

The development of new therapeutic strategies for cholesterol control is an important issue, as also proved by numerous papers recently published. Among the most innovative therapeutic strategies those based on nutritional or pharmacological interventions modulating high-density lipoprotein (HDL) metabolism and reverse cholesterol transfer are the most promising ones.

Cholesterol synthesis and transport

Cholesterol is an essential structural component of mammalian cell membranes and plays crucial roles in intracellular transport, cell signalling, and nerve conduction. In mammals, it serves as the precursor for bile acids, vitamin D, and steroid hormones in liver, skin, and steroidogenic tissues, such as adrenal gland, testis, and ovary. Proper regulation of cholesterol homeostasis in the body is important for human health. Cholesterol homeostasis in the body is maintained mainly by de novo synthesis, intestinal absorption, and biliary excretion. Liver has a central role in cholesterol balance. In fact, to the liver arrive dietary cholesterol and cholesterol from extra-hepatic tissues, and the de novo synthesis is mainly localized in such an organ. Dietary cholesterol is 20% of total cholesterol while the remaining 80% is newly synthesized cholesterol. The excretion of cholesterol (1g/day), mainly as bile salts, is also liver mediated since mammals are unable to hydrolyze cholesterol. Cholesterol synthesis takes place in four stages and it is a very energetic expensive process requiring 18 acetyl-CoA, 18 ATP, 16 NADH, and 4 O2 molecules1.

Cholesterol transport in the blood is mediated by lipoproteins. Chylomicrons and very low-density
lipoproteins (VLDL) mediate the delivery of triglycerides to the peripheral tissues, while low-density lipoproteins (LDL) transport cholesterol to peripheral tissues and the smaller HDL transport cholesterol back to the liver. Cholesterol transported via LDL to tissues is called “bad cholesterol” while the reverse HDL-transported cholesterol is called “good cholesterol”.

Hypercholesterolemia is a major cause of developing atherosclerosis and atherosclerosis-induced conditions, such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease. A major lipid risk factor is a reduced plasma level of HDL-cholesterol, i.e. less than 40 and 50 mg/dL for men and women, respectively. Levels of LDL-cholesterol and triglycerides are less risk predictive.

Drugs available for the control of human cholesterol

For the control of human cholesterol five classes of drugs are on the market: inhibitors of cholesterol synthesis (statins), bile acids binding resins, niacin, fibric acid derivatives, and the cholesterol absorption inhibitor ezetimibe. These drugs provide benefit in patients across the entire spectrum of cholesterol levels primarily reducing levels of LDL-cholesterol. However, these drugs are not always successful, and all of them have severe adverse effects. New strategies for cholesterol control are under investigation and the search for new targets involves both pharmacological and nutritional approaches, mainly focused on HDL metabolism and in particular on RCT, and/or on cholesterol biliary excretion and intestinal secretion/absorption.

HDL function and metabolism

HDL have pleiotropic functions among which the anti-atherosclerotic, anti-inflammatory, antioxidant, and antithrombotic are the most important ones.

Anti-atherosclerotic effect—HDL administration inhibits development of fatty streaks and induces regression of atherosclerotic lesions in cholesterol-fed rabbits. HDL can deplete atherosclerotic plaques through their ability to promote efflux of cholesterol from lipid-loaded macrophages.

Anti-inflammatory effect—In animal models, HDL reduce leukocyte homing to arterial endothelium and increased HDL levels have been associated with a decrease in the blood concentration of pro-inflammatory molecules both in animal models and patients.

Antioxidant effect—HDL inhibit either the enzymatic or non-enzymatic oxidation of LDL and exert indirect antioxidant effects acting as a “sink” for oxidized products that come from LDL transporting the oxidized lipids to the liver for elimination.

Antithrombotic effect—HDL are able to reduce thrombin-induced tissue factor expression in endothelial cells thus inhibiting platelet activation.

HDL precursor is lipid-free apolipoprotein A-I (ApoA-I). ApoA-I is produced by liver and intestine or is released from triglyceride rich proteins (VLDL and chylomicrons). Initial lipidation of ApoA-I by cholesterol and phospholipid efflux from hepatocytes is mediated by the ABC transporter ABCA1 to form discoidal HDL. Lecithin cholesterol acyl-transferase (LCAT) mediates esterification of cholesterol and generates spherical particles (HDL₃) that continue to grow upon ongoing cholesterol esterification and phospholipid transfer protein (PLTP)-mediated particle fusion, and receiving further cholesterol and phospholipids from liver transporters ABCA1 and ABCG1. The so formed mature HDL₄ particles, via cholesteryl ester transport protein (CETP), are ready to transfer cholesteryl esters (CE) to VLDL, which are then transformed in LDL. LDL can undergo receptor-mediated endocytosis in the liver or can migrate to peripheral tissues. HDL can also deliver CE to the liver through scavenger receptor B1(SR-B1).

Reverse cholesterol transfer (RCT)

ApoA-I can scavenge cholesterol and CE from peripheral tissues such as macrophages or endothelial cells, and transports them back to the liver (Fig. 1b). The endothelial binding, uptake, and transport of ApoA-I and HDL are modulated by ABCA1, ABCG1, and SR-BI. This process is called “reverse cholesterol transfer (RCT)”. HDL particles are critical acceptors of cholesterol from lipid-loaded macrophages thereby participating in the maintenance of net cholesterol balance in the arterial wall and in the reduction of pro-inflammatory responses by arterial cholesterol-loaded macrophages. The pathways that regulate HDL-mediated macrophage cholesterol efflux and cholesterol disposition involve cell membrane-bound transporters, plasma lipid acceptors, plasma proteins and enzymes, and hepatic cellular receptors (SR-BI). SR-BI is a multifunctional receptor that mediates bidirectional lipid transport in the macrophage, depending on the content of cholesterol in lipid-loaded macrophages. A well
established role for SR-BI in cholesterol trafficking involves selective uptake of CE from mature HDL by the liver. In foam cells, HDL can remove the excess of cholesterol by means of multiple pathways. The ABCG1 pathway does not contribute to efflux, the SR-BI pathway is relatively important, while the ABCA1 pathway is predominant\textsuperscript{11}. 

**Ecto ATPase/synthase and HDL internalization**

A high-affinity HDL receptor for ApoA-I was identified on the surface of hepatocytes. This receptor is identical to the β-subunit of ATPase/synthase, a principal protein complex of the mitochondrial inner membrane\textsuperscript{12}. The mitochondrial ATPase/synthase, also named $F_0F_1$ATPase/ synthase or complex V, is a 600 kDa multisubunit complex localized in the inner mitochondrial membrane that uses the energy contained in the transmembrane proton gradient to drive the synthesis of ATP from ADP to Pi\textsuperscript{13,14}. Traditionally, ATPase/synthase is divided into two subcomplexes, the membrane-embedded $F_0$ subcomplex through which the protons flow, and the peripheral $F_1$ subcomplex that carries nucleotide binding sites. ApoA-I binding to ecto $F_1$ATPase stimulates extracellular ATP hydrolysis to phosphate and ADP and the latter activates the purinergic G protein-coupled receptor $P_2Y_{13}$ resulting in HDL holoparticle endocytosis. Exogenous IF\textsubscript{1}, classically known as a natural mitochondrial specific inhibitor of $F_1$ATPase activity, inhibits ecto $F_1$ATPase activity and decreases HDL endocytosis\textsuperscript{15,16} (Fig. 2).

The ectopic presence of the β-subunit of $F_0F_1$ATPase/synthase was confirmed also on the surface of endothelial cells where ApoA-I and HDL exert several athero-protective properties within the arterial wall rather than in the blood stream, including cholesterol efflux from macrophage foam cells. In addition, in endothelial cells, angiotatin, an endogenous angiogenesis inhibitor, binds to and inhibits cell surface $F_0F_1$ATPase/synthase, thus modulating endothelial cell proliferation. On the surface of endothelial cells, the $F_0F_1$ATPase/synthase hydrolyzes ATP upon binding of ApoA-I and the produced ADP stimulates trans-endothelial transport of initially lipid-free ApoA-I and HDL via activation of the $P_2Y_{12}$ receptor. The sequence of events
HDL particle endocytosis. Conversely, IF1 protein density plasma bound to HDL. The main function of CETP is demonstrated also in humans adipose tissue, and macrophages, and circulates in inhibition—CETP is synthesized in liver, spleen, Milano could increase RCT. This effect has been recombinant ApoA-I Milano suggest that ApoA-I by cysteine. Several studies in rabbits that received ApoA-I Milano, arginine in position 173 is substituted but no increase in the risk of heart disease. In 888 hydrolysis, and the ADP generated selectively activates the Image 60x561 to 301x689 To facilitate the exchange of CE and triglycerides to convert VLDL in LDL. CEPT collects cholesterol esters from HDL and exchanges them for triglycerides from chylomicrons, VLDL, and LDL, and vice versa. Therefore, the net effect of CETP is pro-atherogenic.

At present, three CETP inhibitor drugs are under clinical trials. Torcetrapib showed an important effect in the raise of HDL in animal models and in humans. In a recent phase III clinical trial\textsuperscript{22,23}, a higher overall cardiovascular mortality was observed in the torcetrapib group despite an efficient HDL increase, which was related to sodium raise, potassium decrease, and blood pressure increase. For these reasons, the study was stopped\textsuperscript{24}. Whereas, preclinical animal models\textsuperscript{25,26} and phase I clinical trials\textsuperscript{27} demonstrated that CETP inhibitors dalcetrapib and anacetrapib can increase HDL without adverse effects on blood pressure. Dalcetrapib is highly specific for the interaction of cysteine 13 in CETP and offers the advantage of a high pharmacological potency, having an IC\textsubscript{50} of 0.4-10 µM for the inhibition of CETP (compared to an IC\textsubscript{50} of 19-79 µM for torcetrapib), while anacetrapib, the third CETP inhibitor, has an IC\textsubscript{50} of 15-57 µM\textsuperscript{25}.

Liver X Receptor (LXR) agonists—The expression of transporters ABCA1 and ABCG1 is increased by LXR transcription factors, which play a pivotal role in modulating cholesterol efflux by both ABC transporters. There are two LXR isoforms: LXRβ, ubiquitously expressed, and LXRα distributed in a tissue-specific fashion, mainly in liver and tissues involved in lipid metabolism. LXRα is activated by specific oxidized forms of cholesterol (oxysterols) such as 27(S)-hydroxysterol and by certain intermediates in cholesterol biosynthesis (Fig. 3).

Analysis of LXRα-deficient mice has revealed a broad role of this receptor in the regulation of genes involved in lipid homeostasis\textsuperscript{28}. For instance, in mice LXRα agonists reduce cholesterol absorption in the intestine due to the up-regulation of ABCG5 and ABCG8, which increase the efflux of cholesterol thereby limiting its absorption by intestinal cells\textsuperscript{29}. Most interestingly, ABCA1, a key transporter in the efflux of cholesterol and phospholipids from macrophages, is a direct target of LXRα agonists\textsuperscript{30}. In fact, treatment with endogenous LXRα agonists is able to increase the RCT from macrophages and foam cells, thus increasing cholesterol biliary excretion\textsuperscript{31}. At the same time, synthetic LXRα agonists, such as the potent LXRα agonist T0901317, are able to

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Fig. 2—Model of the leading hypotheses regarding the ecto F\textsubscript{1}\textsubscript{F\textsubscript{2}}ATPase/synthase-mediated HDL endocytosis by hepatocytes. ApoA-I binding to ecto F\textsubscript{1}ATPase stimulates extracellular ATP hydrolysis, and the ADP generated selectively activates the nucleotide receptor P\textsubscript{2}Y\textsubscript{13} and subsequent G-protein signaling that controls holo-HDL particle endocytosis. Conversely, IF1 protein which inhibits the ecto F\textsubscript{1}ATPase activity down regulates holo-HDL particle endocytosis. Ecto F\textsubscript{1}ATPase, cell surface F\textsubscript{1}F\textsubscript{2}ATPase/synthase; Apo, apolipoprotein; HDL, high-density lipoproteins.

Emerging strategies to increase HDL

Two important reviews regarding steps of HDL metabolism and RCT as new pharmacological strategies have been recently published. The most promising approaches regard genetic modification of ApoA-I, inhibition of CETP, and liver X receptor α (LXRα) agonism\textsuperscript{18,19}.

Genetics of HDL: ApoA-I Milano—ApoA-I Milano (ETC-216) is a naturally occurring mutant of ApoA-I first described in 1980\textsuperscript{20}. Paradoxically, carriers of this mutation have very low HDL cholesterol levels, but no increase in the risk of heart disease. In ApoA-I Milano, arginine in position 173 is substituted by cysteine. Several studies in rabbits that received recombinant ApoA-I Milano suggest that ApoA-I Milano could increase RCT. This effect has been demonstrated also in humans\textsuperscript{21}.

Cholesterol ester transport protein (CEPT) inhibition—CEPT is synthesized in liver, spleen, adipose tissue, and macrophages, and circulates in plasma bound to HDL. The main function of CETP is
SR-BI blockade by ITX5061, a molecule initially characterized as a p38 mitogen-activated protein kinase (MAPK) inhibitor, increases HDL although transiently.35

**Ecto F_0F_1ATPase/synthase inhibitors**—The clinical relevance of ecto-F_0F_1 complex inhibition has been poorly explored. At present, niacin is the only drug that has been involved in ecto-ATPase/synthase regulation. This antilipidemic drug inhibits cell surface expression of the ATPase/synthase β-subunit, leading to reduced hepatic removal of HDL. The exact mechanism of this effect is still unclear, however preliminary observations show that niacin had no effect on mRNA levels of ATPase/synthase β-subunit and over expression of ATPase/synthase by cell transfection did not result in increased surface presence of β-subunit.36 These data suggest that niacin may affect some yet unknown step(s) in the translocation of β-subunit between mitochondria and cell surface.

### Control of intestinal cholesterol absorption and re-absorption: new pharmacological and nutritional strategies

**Pharmacological approach**—Biliary sterol secretion as well as intestinal secretion of neutral sterols supposedly represent the final steps in RCT, but the relevance of these pathways has not been deeply clarified. Recently, a novel transport route for cholesterol has been reported, showing that the intestine contributes significantly to fecal neutral sterol secretion in a LXRα-dependent fashion. Transintestinal cholesterol secretion is a specific process observed throughout the small intestine which depends on the presence of a cholesterol acceptor and is strongly stimulated by bile salts and phospholipids. In mice the contribution of this pathway to cholesterol excretion is approximately twice that of the biliary pathway confirming that intestine plays a significant role in removal of cholesterol from the body in animal models.37 The enterocyte transport is independent on biliary cholesterol secretion but the stimulating effect of LXRα agonists on RCT requires biliary cholesterol secretion. These findings have implications for therapies against atherosclerotic cardiovascular diseases targeting the RCT path.38

Recent studies have demonstrated that Niemann-Pick C1-Like 1 (NPC1L1) protein is the key player in both dietary cholesterol absorption and biliary re-absorption. Since cholesterol de novo biosynthesis
is an energy-consuming process requiring substantial energy input, the body has evolved to take up readily available cholesterol molecules from the gut lumen\(^\text{30}\). NPC1L1, a trans membrane protein highly expressed in mammal enterocytes, has been shown to play a crucial role in cholesterol absorption. In addition, significant expression of NPC1L1 has been observed also in human liver\(^\text{39}\). NPC1L1 is the molecular target of ezetimibe, an inhibitor of cholesterol absorption that has been approved for the treatment of hypercholesterolemia. However, ezetimibe has limits and side effects such as elevation of serum aminotransferase levels and hepatitis. ABCG5/G8 hetero-dimer is also present in the apical membrane of both hepatocytes and enterocytes, where it mediates sterol efflux to bile or intestinal lumen (Fig. 4).

Based on the newly discovered molecular mechanism of NPC1L1-mediated cholesterol uptake, several new strategies can be approached for developing new cholesterol-lowering drugs\(^\text{38}\) including: 1. compounds that can block the cholesterol-regulated NPC1L1 recycling between plasma membrane and endocytic recycling compartment, which may block the cholesterol uptake as well; 2. compounds that can compete with cholesterol for binding to NPC1L1-N-terminal domain which could also be cholesterol-absorption inhibitors; 3. compounds that can interfere with flotillins, principal protein components of lipid rafts (i.e., sphingolipid- and cholesterol-rich membrane microdomains, which serve as organizing centres in a variety of signaling pathways and influence membrane fluidity and cellular cholesterol trafficking) which play an essential role in the formation of NPC1L1–flotillin–cholesterol-microdomains\(^\text{39}\).

**Nutritional approach**—In the cholesterol intestinal trafficking regulation, nutrition can give an important therapeutic contribution. Fibres, phytosterols, and probiotics have a codified role in fighting “bad cholesterol”\(^\text{40}\).

(i). Fibres: Increased consumption of viscous soluble dietary fibres (10-25 g/day) impairs absorption of dietary cholesterol and re-absorption of bile acids. Bacterial fermentation of soluble fibres results in short chain fatty acids that may inhibit cholesterol synthesis with minimal laxative side effects\(^\text{40}\).

(ii). Phytosterols: Phytosterols are non nutritive compounds that may be found in a great variety of different food products. Many studies have demonstrated their ability to reduce blood cholesterol levels\(^\text{41}\) in hyper- and normo-cholesterolemic subjects. Investigators report that phytosterol intakes of 2 to 3 g/day reduce LDL-cholesterol levels by about 10% in human subjects\(^\text{42}\).

Their chemical structure differs from that of cholesterol because of additional alkyl substituents at C-24 and/or a double bond at C-22. The action mechanism of phytosterols is based on their ability to reduce cholesterol absorption through co-precipitation of cholesterol or competition for space in mixed micelles, although several findings suggest additional mechanisms.

Dietary cholesterol intruded into enterocytes via NPC1L1 is esterified and then absorbed into the lymph with chylomicrons or re-extruded as free cholesterol into the gut lumen by ABCG5/G8 or loaded onto HDL by ABCA1. CYP27 (sterol 27-hydroxylase) generates 27-hydroxycholesterol (27OH-C) which activates LXR\(\alpha\) and enhances basolateral ABCA1 but not apical ABCG5/G8 expression. Phytosterols interfere with 27OH-C formation by CYP27 and the self-priming of cholesterol absorption despite rapid extrusion by ABCG5/G8\(^\text{43}\) (Fig. 5).

In most cells, ABCA1 acts as a general cholesterol exporter protecting the cell from surplus free cholesterol. This is signaled by oxysterols activating LXR\(\alpha\). In polarized enterocytes, ABCA1

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**Fig. 4**—The function of NPC1L1 protein in vivo. NPC1L1 is located at the brush border membrane of the enterocyte and canalicular membrane of the hepatocyte. Water-insoluble cholesterol is delivered to the brush border membranes by bile salt micelles. NPC1L1 mediates cholesterol inward transport from the brush border membrane to the intracellular compartment. The majority of cholesterol taken up into the enterocyte by NPC1L1 is subsequently esterified, packaged into chylomicrons, and then secreted into lymph. The chylomicrons are finally transported to the liver via blood circulation. This process is called the enterohepatic circulation of endogenous cholesterol. Liver NPC1L1 facilitates biliary cholesterol re-absorption into hepatocytes. The ABCG5/G8 heterodimer is also present in the apical membrane of both hepatocytes and enterocytes, where it mediates sterol efflux to bile and intestinal lumen, respectively. NPC1L1, Niemann-Pick C1-Like 1 protein; ABCG5/G8, ATP-binding cassette transporter G5/G8.
Mechanism of ABCA1 expression. Cholesterol intrudes into enterocytes via NPC1L1, then can be absorbed in the lymph or extruded as free cholesterol by ABCG5/G8 or loaded by ABCA1 onto HDL. CYP27-generated 27OH-cholesterol activates LXRα and enhances basolateral ABCA1 expression. Phytosterols competing with the formation of 27OH-cholesterol reduce the expression of ABCA1 gene. NPC1L1, Niemann-Pick C1-Like 1 protein; ABCG5/G8 and ABCA1, ATP-binding cassette transporters G5/G8 and A1; CYP27, sterol 27-hydroxylase; LXRα, liver X receptor α; 27HO-Chl, 27-hydroxycholesterol.

As an effector of basolateral systemic cholesterol absorption. Its expression is enhanced by 27OH-C generated during the influx of dietary cholesterol, whereas apical ABCG5/G8 expression is unchanged in humans. Phytosterols reduce 27OH-C formation by competitive inhibition of CYP27 thus preventing self-priming component of systemic cholesterol absorption. This favours sterol re-excretion into the gut lumen by ABCG5/G8. Consequently, local LXRα antagonism by phytosterols reduces fractional cholesterol absorption by the enterocyte ABCA1 pathway.

(iii). Probiotics: Probiotics are defined as live microorganisms that, when administered in adequate amounts confer a health benefit to the host. Microorganisms used as probiotics are mainly bacterial strains of Lactobacillus (L. acidophilus, L. casei, L. plantarum, L. reuteri, L. rhamnosus, and L. salivarius) and Bifidobacterium (B. bifidum, B. breve, B. longum, and B. lactis). Less commonly used are the bacterial strains of Bacillus (B. subtilis and B. cereus var. toyoi), Enterococcus (E. faecium and E. faecalis), Streptococcus (S. thermophilus and S. salivarius), Lactococcus and Escherichia among others. The yeast Saccharomyces boulardii is also used as a human probiotic, in the forms of capsules or powders rather than in food form. Probiotics should be safe even when administered in large amounts, they should exhibit dose–response relationships, and they should survive the industrial processes involved in their massive incorporation into foodstuffs. Most of these requirements have already been included in Food and Agriculture Organization (FAO)/WHO guidelines for the evaluation of probiotics for food use.

The use of probiotics is a modern strategy in the prevention and treatment of hypercholesterolemia and numerous mechanisms for hypocholesterolemic effect of probiotics have been hypothesized, based mostly on in vitro evidence. Interaction with bile acids, through reaction of deconjugation catalyzed by bile salt hydrolase enzymes (BSH), is considered as the main mechanism of cholesterol-lowering effects of probiotic bacteria. The mechanism is based on the ability of certain probiotic lactobacilli and bifidobacteria to deconjugate bile acids enzymatically, thus increasing their rates of excretion. Deconjugated bile salts are less soluble and less efficiently reabsorbed from intestinal lumen than their conjugated counterparts, thus resulting in fecal excretion of larger amounts of free bile acids. Numerous Lactobacillus strains are able to deconjugate bile salts and remove cholesterol to varying degrees in vitro, but not all strains with high deconjugating activity are able to effectively remove cholesterol in vivo. These results suggest the existence of other mechanisms involved in cholesterol removal, including cholesterol binding to cell walls of probiotics, assimilation and incorporation of cholesterol into the cellular membranes of bacteria, co-precipitation of cholesterol with deconjugated bile acids, conversion of cholesterol into coprostanol, and production of short-chain fatty acids (SCFA).

It has been shown that cholesterol could be assimilated by L. acidophilus (strain ATCC 43121) and that assimilated cholesterol was not metabolically degraded. The same study demonstrated that cells grown in presence of cholesterol micelles and bile salts were more resistant to lysis by sonication than those grown in their absence. Likewise, strains of Lactobacillus and Bifidobacterium, grown in media containing cholesterol, appeared more resistant to sonication and enzymatic lysis compared to those grown without cholesterol. In addition, probiotic bacteria ferment the food-derived indigestible carbohydrates (soluble fibres) in the human gut thus increasing production of short chain fatty acids, such as acetate, propionate, and butyrate. The relative proportions of these acids depend on the substrate. Acetate has been shown to increase the cholesterol synthesis, while propionate had the opposite effect.
Therefore, substrates that can decrease the acetate to propionate ratio, such as polyfructans (e.g., inulin and oligofructose) may reduce serum lipids.

Huang et al. identified NPC1L1 protein as a key player in cholesterol absorption and a promising target for cholesterol-lowering by probiotics. It has been shown that L. acidophilus ATCC 4356 reduced NPC1L1 gene expression and inhibited the cellular uptake of micellar cholesterol in Caco-2 cells. Soluble effector molecules secreted by L. acidophilus ATCC 4356 appeared to be responsible for this effect. Based on the finding that the expression of NPC1L1 gene is down regulated by LXR activators in the intestine, Huang et al. examined whether L. acidophilus ATCC 4356 mediated this effect at least partly through LXR activation. They demonstrated that ATCC 4356 up regulated the expression of LXR in a dose- and time-dependent manner. In addition, when LXR was depleted by siRNA in Caco-2 cells, NPC1L1 expression was no longer decreased and ATCC4356 could not significantly reduce micellar cholesterol uptake. As a whole, these findings suggest the intriguing possibility that it may be possible to modify NPC1L1, a central player in cholesterol homeostasis, by manipulating the gut microbiota.

Conclusion

Presently available anti-cholesterolemic drugs such as statins mainly act on LDL modulation and their clinical use is significantly limited by incomplete efficacy and severe adverse effects. This encourages active search for innovative therapeutic strategies based on both pharmacological and nutritional interventions. Current research is focused on the emerging HDL-mediated cardio-protective mechanisms, regarding in particular both the pharmacological and nutritional regulation of RCT. However, the beneficial therapeutic effects of raising HDL proved in experimental animals are still difficult to confirm in humans.

Compared to the pharmacological approach the nutritional ones could be not only less expensive but also safer in controlling the terminal part of RCT, i.e. cholesterol intestinal absorption and re-absorption. Soluble fibres, phytosterols, and probiotics used alone or in association can modulate expression of intestine and liver ABC transporters and NPC1L1 which have been demonstrated to be critically involved in the cholesterol balance in humans.

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