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ABC Transporters and Sterol Absorption

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Abstract: Recent molecular studies, in particular investigations of subjects with monogenic disorders of lipoprotein metabolism and studies of induced-mutant mice, have increased the understanding of intestinal sterol absorption. Some of these genes encode adenosine triphosphate [ATP] binding cassette (ABC) transporters that transport dietary cholesterol from enterocytes back out to the intestinal lumen, thereby limiting the amount of cholesterol absorbed. ABC transporters also provide an effective barrier against the absorption of plant sterols, which are normally not absorbed in significant quantities by humans. This mechanism was clarified by the discovery that defects in two adjacent genes encoding ABC transporters are the molecular basis of sitosterolemia, a rare autosomal recessive disease in which plant sterols are absorbed due to failure of intestinal barrier to their absorption. Furthermore, recent experiments performed in induced-mutant mice have solidified the importance of these transporters in intestinal sterol absorption. Together with new developments in the biology of bile acids, sterol absorption is providing interesting directions for metabolism research. In addition to elucidating some of the molecular mechanisms of sterol absorption, these recent findings may lead to new therapeutic options to treat hypercholesterolemia and to help patients at risk of vascular disease reach ever-more stringent target levels.

Key Words: Sterols, intestinal absorption, nuclear hormone receptors, ABC transporters, sitosterolemia, cholesterol esters.

INTRODUCTION

Sterols are a class of lipids comprised of four carbon rings. They are essential components of animal and plant cell membranes. In humans, sterols are needed for normal physiologic processes, such as synthesis of steroid hormones and vitamin D, and also of oxysterols and bile acids, which activate nuclear hormone receptors involved in sterol metabolism. Cholesterol is also required for activation of sonic hedgehog, which is involved with forebrain patterning. Cholesterol is the sterol synthesized and utilized by animals, whereas sitosterol, campesterol and stigmasterol are some of the major sterols found in plants. While structurally similar, plant sterols differ from cholesterol by the addition of a methyl or ethyl group. These side-chains impart functional specificity to the particular sterol, of which one significant consequence is prevention of the efficient absorption of certain sterols (especially plant sterols) in the human intestine [1]. Excess sterol production or absorption is of interest because of its potential effect on plasma lipoprotein concentrations, which are important determinants of atherosclerosis [2,3]. Thus, understanding the factors involved in the intestinal absorption of sterols might help to specify newer molecular targets, which could lead to better pharmacological treatments for hypercholesterolemia.

Despite the fact that sterol absorption has been studied biochemically for years, most of the molecular mechanisms involved had been poorly understood until recently. However, relatively recent and exciting research findings have advanced our understanding of intestinal sterol absorption at the molecular level. In this review, some of these findings will be discussed, specifically, the identification of ATP-binding cassette (ABC) transporters as key sterol transport molecules in enterocytes.

OVERVIEW OF STEROL ABSORPTION

The absorption of sterols in the human diet is a complex, multi-step process (see Fig. **1**). A crucial component determining absorption is solubility of sterol within the gastrointestinal tract, with bile acids (BA) assuming a leading role. The basic principles underlying sterol solubility in bile have been understood for more than three decades [2,3], but many of the molecular mechanisms that underlie these pathways have only come to light recently [for detailed reviews, see 4-8]. Within the stomach, dietary components form a lipid-water emulsion that is stabilized by phospholipids. In the duodenum, pancreatic lipase hydrolyses the triglycerides (TG) at the surface of the fat emulsion. Bile acid (BA) secreted from the gall bladder forms micelles with free fatty acids (FFA), mono- and di-acylglycerols, lysophospholipids, and cholesterol. BA is secreted and subsequently re-absorbed through specific mediators and permits absorption of lumenal free cholesterol, which is almost entirely insoluble in water and poorly soluble in bile salt solutions. However, free cholesterol is soluble in phosphotidylcholine (PC) lamellar bilayers, which can then be solubilized by BA forming mixed micelles that carry the free cholesterol into solution. In the jejunum, ~40% of the cholesterol is absorbed via passive diffusion into epithelial cells. Within enterocytes, processing by acyl coA:cholesterol acyltransferase, also called sterol O-acyltransferase (SOAT), prepares cholesterol esters and triglycerides for assembly with apolipoprotein (apo) B-48 into chylomicrons, which

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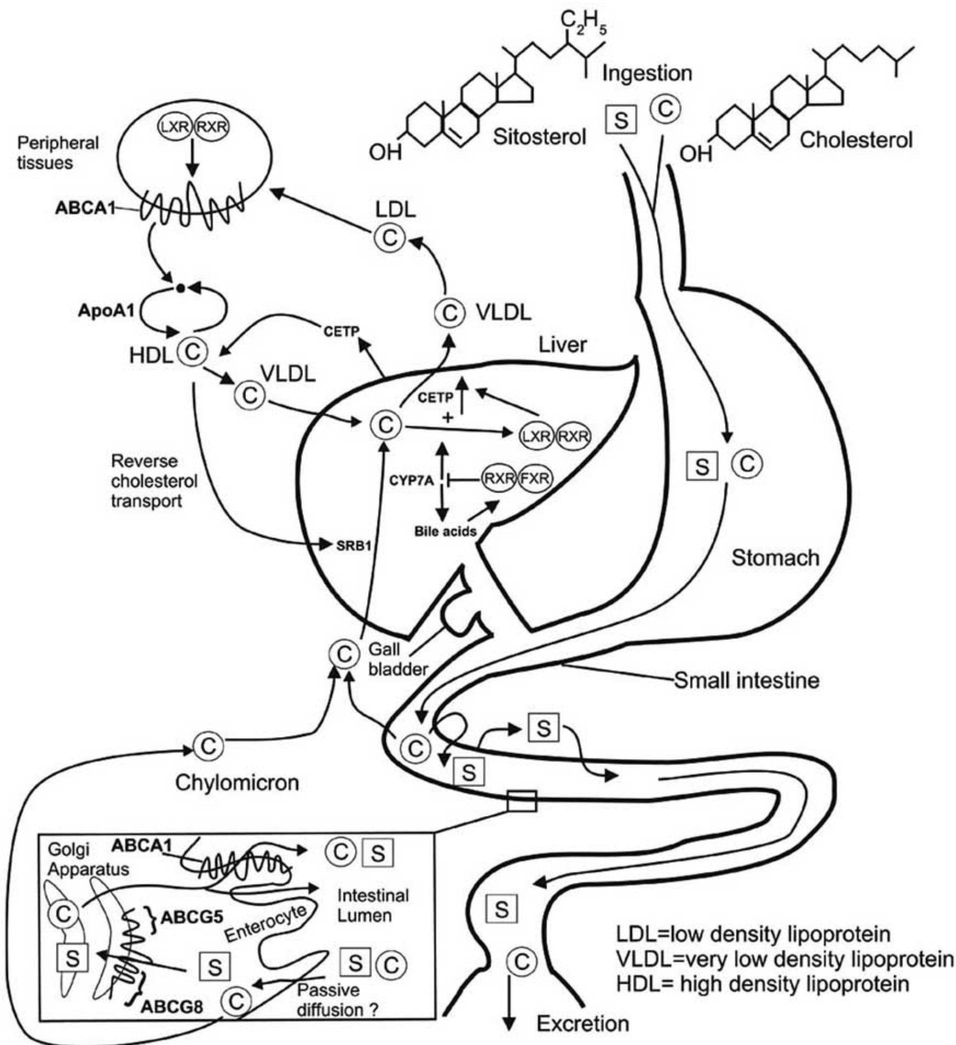

Fig. (1). Sterol absorption: a brief overview. Dietary sterols include plant sterols, such as sitosterol (S), and animal sterols, such as cholesterol (C). Ingested sterols enter epithelial cells of the gastro-intestinal tract (enterocytes), perhaps by passive diffusion. The inserted rectangle shows the passage of C and S through the enterocyte. Under regulatory control of liver X receptor (LXR), ABCG5 and ABCG8 dimerize to form a sterol transporter within the cell surface plasma membrane and probably with membranes of intracellular organelles. These transporters actively transport S, but also some C, back into the gut lumen, thus limiting net sterol absorption. The ABCA1 transporter may also participate in this process; ABCA1 pumps C from enterocytes into the intestinal lumen and may also function on the basal side of intestinal cells, although this is controversial. ABC transporters in the intestine allow only a very small percentage of plant sterols into the blood stream. Absorbed sterols are packaged into chylomicrons, which are ultimately catabolized by the liver. In the liver, C and S may be packaged for transport to peripheral tissues by very-low density lipoprotein (VLDL) and low-density lipoprotein (LDL) or converted into bile acids (BA) and transported into the bile. In peripheral cells, the ABCA1 transporter delivers C to high-density lipoprotein (HDL) for transport back to the liver. Nuclear hormone receptors (LXR and RXR) coordinate reverse cholesterol transport from peripheral tissues to the liver by activating expression of ABCA1 in cholesterol loaded peripheral cells (notably macrophage foam cells), leading to enhanced efflux of C onto apo A-I, resulting in the formation of mature HDL. HDL may bind to scavenger receptor BI (SRBI), promoting selective uptake of cholesterol into the liver. In plasma, cholesterol ester transfer protein (CETP), whose expression in liver is regulated by LXR, transfers C to VLDL. In the liver, C may be converted into BA by cholesterol 7-alpha hydroxylase (CYP7A1 encoded by *CYP7A1*) and then be excreted into the gall bladder. Also, the BA-activated receptor FXR may repress CYP7A1 expression, preventing the upregulation of CYP7A1 by LXRs and leading to repression of bile salt synthesis.

represent the main exogenous class of lipoprotein that is secreted into lymph and ultimately into plasma. Endothelial-bound lipoprotein lipase (LPL), with its co-factor apo C-II, liberates FFA from chylomicrons, and remodels the particles into smaller, denser, more cholesterol ester-rich chylomicron remnants. The chylomicron remnants are endocytosed by remnant receptors on hepatocytes using apo E as the main ligand.

ABC TRANSPORTERS

Recent experimental evidence indicates a key role in sterol absorption for enterocytic ABC transporters, which are members of a family of >50 integral membrane proteins that actively transport substances across cell membranes using energy acquired from ATP [9]. Mutations in the genes encoding ABC transporters have been implicated in several diseases, including cystic fibrosis (transport of chloride ions) and adrenoleukodystrophy (transport of very-long-chain fatty acids) [9]. Mutations in *ABCC6* cause pseudoxanthoma elasticum, but may also be associated with variation in plasma lipoproteins [10]. A partial list of human genetic disorders that have been associated with genetic variation in ABC transporters is shown in Table **1**.

Another ABC transporter, ABCA1 (human protein in capital letters), has been recently shown to play an important role in cellular cholesterol and plasma lipoprotein metabolism [11-13]. In particular, defects in *ABCA1* (human gene in capitalized italics) prevent the reverse transport of cholesterol from peripheral tissues back to the liver, resulting in Tangier disease, a condition that is characterized by the accumulation of cholesterol esters in peripheral tissues. ABCA1 also plays a key role in regulating the absorption of dietary cholesterol, by directing cholesterol from enterocytes back out to the intestinal lumen [14].

ABC TRANSPORTERS AND BILIARY METABOLISM

The secretion of biliary lipids is regulated to a large degree by ABC transporters, and this could serve as a separate topic for an extensive review. In short, many ABC transporters, such as multidrug resistance proteins, have been implicated as being important in biliary secretion, which may not be surprising given the importance of biliary excretion as a terminal pathway for drug metabolism. For instance, targeted disruption of the *ABCB4* gene encoding the multiple drug-resistant protein MDR2 has been shown to prevent the secretion of PC and cholesterol into bile and furthermore was demonstrated to cause liver pathology [15]. An attempt to rescue biliary PC in this model with high rates of bile salt infusion could not satisfactorily restore biliary PC [16], thus implicating MDR2 as the PC transporter. Another example is the *ABCB11* gene, which encodes the bile salt export pump (BSEP). BSEP is located at the bile canniliculus and regulates most of the BA transport from the hepatocyte into the bile cannilicular lumen [17-20]. Human mutations in both *ABCB4* and *ABCB11* give rise to progressive familiar intrahepatic cholestasis (see Table **1**) [21,22].

ABCG5, ABCG8 AND STEROL ABSORPTION

In addition to transporting cholesterol, ABC transporters appear to be involved in the metabolism of plant sterols.

Table 1. Selected List of ABC-transporters that have been Associated with Mendelian (Monogenic) Diseases and/or Complex Diseases Phenotypes

Gene	Mendelian disorder	Complex disease	OMIM
ABCA1	Tangier disease, FHDLD		600046
ABCA4	Stargardt/FFM, RP, CRD, CD	AMD	248200
ABCB1	Ivermectin susceptibility	Digoxin uptake	171050
ABCB2	Immune deficiency		170260
ABCB3	Immune deficiency		170261
ABCB4	PFIC3	ICP	171060
ABCB7	XLSA/A		300135
ABCB11	PFIC2		603201
ABCC2	Dubin-Johnson Syndrome		601107
ABCC6	Pseudoxanthoma elasticum		603234
ABCC7	Cystic Fibrosis, CBAVD	Pancreatitis, bronchiectasis	602421
ABCC8	FPHHI		600509
ABCD1	ALD		300100
ABCG5	Sitosterolemia		605459
ABCG8	Sitosterolemia		605460

Abbreviations: OMIM, Online Mendelian Inheritance in Man reference number; FHDLD, familial hypoalphalipoproteinemia; FFM, fundus flavimaculatis; RP, retinitis pigmentosum; CRD, cone-rod dystrophy; AMD, age-related macular degeneration; PFIC3, progressive familial intrahepatic cholestasis type 3; PFIC2, progressive familial intrahepatic cholestasis; ICP, intrahepatic cholestasis of pregnancy; XLSA/A, X-linked sideroblastosis and anemia; CBAVD, congential bilateral absence of the vas deferens; FPHHI, Familial persistent hyperinsulinemic hypoglycemia of infancy; ALD, adrenoleukodystrophy. Table modified from reference [59].

Normally, the intestine provides an efficient barrier against the absorption of plant sterols, i.e., <5% of dietary plant sterols are absorbed, compared with 40–60% of dietary cholesterol [1]. Because plant sterols are also preferentially removed by the liver and excreted into bile, plasma concentrations of plant sterols in most humans are low [1]. Defense against the absorption of plant sterols is disrupted in sitosterolemia, a rare autosomal recessive disorder characterized by the accumulation of large amounts of plant sterols in most tissues [1]. About one person in one million suffers from sitosterolemia, in which the mechanism that distinguishes between absorbed sterols is defective. Patients with sitosterolemia develop atherosclerosis at an early age, and also develop tendon and tuberous xanthomas [1,23]. It has been postulated that increased absorption of dietary cholesterol is associated with the elevated plasma cholesterol levels. As a result, many sitosterolemia patients develop coronary heart disease at an early age. The genetic defect was localized to chromosome 2p21 in 1998 [24], and two groups independently identified the gene as a member of the ABC transporter family [25,26]. Patel and colleagues used a traditional positional cloning and physical mapping approach to identify the sitosterolemia gene mutations [26]. Berge, Hobbs and colleagues [25] were aided by the observation that activation of the liver X receptor (LXR)- retinoid X receptor (RXR) heterodimer increased the *ABCA1* expression in the small intestine and enhanced the efflux of cholesterol from enterocytes [14]. They hypothesized that the sitosterolemia gene(s) may be a transporter protein whose expression could also be induced by LXR. Using DNA microarrays, Berge, Hobbs and colleagues [25] identified genes that had increased expression in enterocytes in response to a synthetic LXR ligand.

Both groups have found that individuals with sitosterolemia have a mutation in one of two genes, namely *ABCG5* and *ABCG8*, which encode for sterolin-1 and sterolin-2 respectively [25,26]. Several different mutations that cause sitosterolemia have been identified in *ABCG8* and *ABCG5*. Their causative role in sitosterolemia implies that the ABCG5 and ABCG8 transporters have a role in pumping plant sterols into the gut lumen. These genes each contain 13 exons, each span ~28 kb, are transcribed in opposite directions and appear to be subject to similar regulation [25]. Each gene actually encodes a half-ABC transporter, which suggests that the gene products join to form a full, active transporter [27]. This mechanistic model is suggested by the fact that mutations in both genes in a single individual have not yet been reported. Co-expression of both gene products is required for the movement of these half-receptors into the Golgi and onto the apical surface of the cell. *ABCG5* and *ABCG8* are expressed in the liver and intestine but not in other tissues [25]. The side chains on plant sterols would appear to be crucial signals affecting the specificity of such active secretion.

Additional evidence of the importance for these half-transporters was provided by transgenic animals overexpressing *ABCG5* and *G8* in which bile cholesterol was markedly increased, and the absorption of added diet cholesterol and plant sterols was markedly decreased [28]. The importance of these half-transporters was affirmed by experiments in which mice *abcg5* and *g8* genes were disrupted had greatly inhibited cholesterol secretion into bile [29]. On a chow diet, the plasma sitosterol was elevated 30-fold in the *abcg5* and *g8* (murine genes in lower-case italics) knockout mice, but the plasma cholesterol was halved. When the *abcg5* and *g8* knockout mice were challenged with a high cholesterol diet, the plasma cholesterol increased 2.4-fold, and liver cholesterol increased more than 6-fold compared to the increase in controls. The *abcg5* and *g8* knockout animals had decreased fecal sterols and increased absorption of plant sterols, similar to patients with sitosterolemia [30].

The role of ABCG5 and G8 in cholesterol transport into bile was further confirmed by findings in the induced mutant mice. Specifically, in the *abcg5* and *g8 -/-* animals, cholesterol in the bile was dramatically decreased, even though PL and BA were secreted normally [30]. In contrast, transgenic mice that overexpressed *abcg5* and *g8* had increased cholesterol in the bile, without changes in PL or BA [29]. The lack of difference in excessive cholesterol secretion by transgenic mice expressing low and high amounts of ABCG5 and G8 could have been caused by a saturation of the acceptors, PC and BA, in the bile canniliculus [31]. Thus, ABCG5 and G8 probably move cholesterol onto acceptors until they are saturated. Small [32] speculated that ABCG5 and G8 acted to easily mobilize cholesterol from cholesterol acceptors, namely intestinal micelles with PL and BA. ABCG5 and G8 may form a transmembrane channel, which binds cholesterol and then directs it into the lumen when ATP is hydrolyzed.

MORE ON BILE ACIDS

Dried extracts of bear bile have been used in oriental folk remedies for hundreds of years [33] and the BAs in these extracts were among the first natural products to be isolated in their native form [34]. The primary BAs in humans are cholic acid and chenodeoxycholic acid. These BAs are formed in the liver through oxidative catabolism of cholesterol and can be further metabolized in the intestine to form the secondary BAs deoxycholic acid and lithocholic acid. These may be conjugated with glycine or taurine to form bile salts, which act as water soluble detergents to aid the transport of cholesterol and fat soluble vitamins through micelle formation. As mentioned above, BAs are required for elimination of hepatic cholesterol, for transport of lipids across the intestine, for stimulation of biliary excretion and for regulation of hepatic cholesterol synthesis. However, in contrast to other biologically active steroids, such as estrogens, androgens and corticosteroids, no receptors for BAs were ever identified using classical biochemistry. As such, research on BAs had focussed mainly on their physical properties as detergents, rather than their potential role as enterohepatic hormones. But all this changed with the discovery that the nuclear hormone receptors LXR and farnesoid X receptor (FXR) are BA receptors. LXR and FXR are now considered to be very important factors that regulate the expression of the components of the sterol absorption pathway.

NUCLEAR HORMONE RECEPTORS AND BILE METABOLISM

A detailed discussion of all the aspects of LXR and FXR is beyond the primary focus of this review. However, given the central role of BA in sterol absorption, it is definitely worth touching upon some aspects of this exciting field within metabolism, especially because of the potential pharmacological implications of these targets. In general, nuclear hormone receptors are intracellular proteins whose primary function is to regulate gene transcription. In the basal state, these receptors are inactive. However, when bound by ligands, these receptors form heterodimers and bind to the promoter regions of genes, with a resultant alteration in the transcriptional activity of the target gene. To date, ~50 nuclear receptors have been identified within the human genome [35,36]. Each of these receptors contains the characteristic DNA-binding and/or ligand-binding domain. However, only some of these nuclear hormone receptors bind to the classical steroid and retinoid hormones, The remainder have been designated as "orphan nuclear receptors". The existence of so many receptors suggests that there are likely to be many additional lipophilic hormones yet to be identified.

Of the nuclear hormone receptor family, FXR and LXR are currently preeminent with respect to allure for the research community. Both LXR and FXR act in conjunction with another nuclear hormone receptor, namely RXR. Further, to affect transcriptional activity, ligand-activated LXR or FXR must heterodimerize with RXR. The natural ligands for LXR are oxysterols, which are oxidized cholesterol derivatives, and the natural ligands for FXR are BA [35-38]. LXR-RXR and FXR-RXR heterodimers control transcription of several genes that actively participate in cholesterol metabolism. For example, the LXR-RXR heterodimer induces the expression of *CYP7A1* encoding cholesterol 7-alpha-hydroxylase, which is the rate-limiting enzyme of the BA synthetic pathway [35-38], thereby increasing BA synthesis. In contrast, the FXR-RXR heterodimer suppresses the expression of *CYP7A1* [14], thereby suppressing BA synthesis.

Pharmacologic studies in mice delineated the importance of nuclear hormone receptors in sterol metabolism and exposed new metabolic mechanisms. In these studies, administration of a synthetic RXR ligand resulted in a dose-dependent inhibition of cholesterol absorption [14]. These pharmacologic studies helped to characterize LXR-RXR– and FXR-RXR–mediated changes in cholesterol homeostasis and indicated that two mechanisms underlie this reduction in cholesterol absorption. First, the FXR-RXR heterodimer suppressed expression of *CYP7A1* and decreased BA synthesis, with a resulting decrease in solubilization and subsequent absorption of dietary cholesterol [14]. Second, activation of the LXR-RXR heterodimer could not prevent the FXR-RXR–mediated suppression of *CYP7A1* expression. However, the activated LXR-RXR heterodimer induced *ABCA1* expression in enterocytes. By increasing *ABCA1* expression, the LXR-RXR heterodimer enhanced the efflux of cholesterol from enterocytes and limited cholesterol absorption [14]. This provided compelling proof of the role of ABCA1 in regulating the dietary sterol absorption. Thus, at least in experimental model systems, pharmacological activation of RXR, LXR and FXR has helped to specify mechanisms that underlie intestinal sterol transport.

A principal biological role of FXR is the maintenance of BA homeostasis [39]. For instance, FXR ligands promote BA synthesis and excretion, with resulting decreases in plasma LDL cholesterol and triglyceride, and concomitant increases in plasma HDL cholesterol. The effects of FXR on its target genes are not uni-directional. FXR binds specific sequences in various gene promoters [40,41], and increases the expression of *PTPN6*, also called *SHP1*, encoding the small heterodimer partner protein [38,41]. In addition to decreasing expression of *CYP7A1* [38,41,42], FXR decreases expression of *CYP8B1* encoding sterol 12-alpha hydroxylase [43,44], of *SLC10A1* encoding a sodium/BA contransporter [45], and *CETP* encoding cholesterol ester transfer protein [46]. BA may repress *CYP7A1* gene expression through FXR-independent mechanisms [47,48]. In contrast, FXR results in increased expression of *ABCB11* encoding BSEP [49], of *FABP6* encoding intestinal bile acid binding protein [50], of *PLTP* encoding phospholipid transfer protein [51,52], and of *APOC2* encoding apo C-II [10], among others. Given this degree of mechanistic complexity, the effect of direct or partial pharmacologic agonist or antagonist activity is not obvious, but is nonetheless the focus of much scientific interest.

RECENT PHARMACOLOGICAL STUDIES

It is worth mentioning exciting recent experiments of LXR agonist administration in animal models, which provide proof-of-concept of the importance of the above pathways in metabolism. For instance, a selective LXR modulator was found to induce *ABCA1* in mouse liver, with a resulting increase in plasma HDL-cholesterol, relatively independent of effects on mediators such as sterol regulatory element binding protein (SREBP)-1c and fatty acid synthase (FAS) [54], although these are considered to be the main lipogenic genes that are stimulated by LXR. In addition, SREBP-mediated activation of cholesterol biosynthesis produces endogenous oxysterol ligands that activate LXR, which in turn activates expression of *ABCG5* and *ABCG8* [55]. The increase in HDL cholesterol seen with an LXR agonist was associated with upregulation of *APOA4* in other mouse experiments [56]. Other experiments in mice have shown that *PPARG* encoding peroxisome proliferator activated receptor (PPAR) gamma in adipocytes is also a target of LXR [57], providing some support for the idea that these LXR agonists might also affect adipogenesis and/or insulin resistance. Also, liver LPL expression was increased by an LXR agonsist in a mouse model [58] and co-administration with a PPAR alpha agonist increased plasma HDL cholesterol. In general, the *in vivo* data to date indicate that treatment with LXR agonists has a net beneficial effect on plasma lipoprotein metabolism, especially increasing HDL cholesterol. New pharmacologic mechanisms and targets for lipoprotein metabolism are important since current evidence-based treatments, such as statin drugs, only reduce events by 25-35%, on average.

SUMMARY

Sterol absorption is complex, but the extent of this complexity was not fully appreciated until recently through the use of powerful experimental tools, such as human molecular genetic analysis and induced-mutant animal models. Elucidation of the genetic basis of sitosterolemia has exposed new pathways for exploration and potential new molecular targets. But the understanding that sterols and bile acids play a key role in nuclear hormone biology and regulation of gene expression is heralds an even more fundamental and exciting new frontier in metabolism, with implications that might range far beyond the intestinal lumen into the cardiovascular, endocrine, ophthalmologic and neurological systems. From the cardiovascular point of view, the new mechanisms specified by these recent discoveries provide new opportunities and targets for drug development to alter sterol metabolism and ameliorate plasma lipids and lipoproteins, whose target levels are becoming more strict in current vascular disease prevention algorithms.

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