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Investigating Sitosterolemia to Understand Lipid Physiology

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Summary

The cholesterol molecule is at the center of the pathophysiology of many vascular diseases. Whole-body cholesterol pools are maintained by a balance of endogenous synthesis, dietary absorption and elimination from our bodies. While the cellular aspects of cholesterol metabolism received significant impetus from the seminal work of Goldstein and Brown investigating LDL receptor trafficking, how dietary cholesterol was absorbed and eliminated was relatively neglected. The identification of the molecular defect a rare human disorder, Sitosterolemia, led to elucidation of a key mechanism of how we regulate the excretory pathway in the liver and in the intestine. Two proteins, ABCG5 and ABCG8, constitute a heterodimeric transporter that facilitates the extrusion of sterols from the cell into the biliary lumen, with a preference for xenosterols. This mechanism explained how dietary xenosterols are prevented from accumulating in our bodies. In addition, this disease has also highlighted the potential harm of xenosterols; macrothrombocytopenia, liver disease and endocrine disruption are seen when xenosterols accumulate. Mouse models of this disease suggest that there are more dramatic alterations of physiology, suggesting that these highly conserved mechanisms have evolved to prevent these xenosterols from accumulating in our bodies.

Keywords

ATP-Binding Cassette Transporters; Phytosterols; Thrombocytopenia; Splenomegaly; Hemolysis; Infertility; Endocrine disruption; Atherosclerosis; Xanthomas; Pseudohomozygous Familial Hypercholesterolemia

Introduction

Management of lipids is vital to normal mammalian physiology. As a major class of circulating lipids, elevated serum cholesterol levels have been shown to be a major risk factor for the development of atherosclerosis and cardiovascular disease. Understanding of sterol metabolism dates back to observations of endogenous cholesterol synthesis in herbivores consuming solely non-absorbable plant sterols [1]. These animals, despite a diet of only plant sterols and negligible cholesterol, show no evidence of accumulation of these ‘xeno’ sterols in their bodies, yet contain substantial amounts of cholesterol. Decades later, we now know that cholesterol homeostasis is regulated by the interplay of dietary cholesterol absorption, endogenous cholesterol synthesis, and biliary cholesterol excretion,

and the exclusion of non-cholesterol sterols (especially plant sterols) from the body. The rare human genetic disorder of Sitosterolemia (OMIM #210250) has allowed critical insights into these biochemical pathways [2]. The disease is caused by mutations in one of two genes, *ABCG5* or *ABCG8*, on human chromosome 2p21 [3]. Affected individuals may suffer from premature cardiovascular disease, hematologic disorders, endocrine disruption, and possibly liver cirrhosis [3]. Through studies of this disorder, it is now known that the two proteins, *ABCG5* and *ABCG8*, function together as key regulators of sterol trafficking and their normal function is critical for the excretion and elimination of xenosterol and excess cholesterol from the body. This review will focus on Sitosterolemia and how investigations of this disease have increased our understanding of lipid and sterol metabolism.

Background: Dietary Sterols

Sterols are important components of the most eukaryotic cell membranes. The 2-part chemical structure of the sterol molecule consists of a cyclopentane phenanthrene ring nucleus that is similar to steroid hormones and bile acids, and a side chain. Primarily found on the plasma membrane lipid bilayer, sterols interact with phospholipid molecules to maintain cell membrane structure and regulate membrane fluidity [4].

In mammals, cholesterol is the major sterol and is the essential molecular precursor for steroid hormones and bile acids and may be involved in cell signaling. Plant sterols (xenosterols) are unique to plants and are not synthesized in mammalian species [5]. Structurally, plant sterols resemble cholesterol in the same ring nucleus, 3 β -hydroxy group, and 8-carbon side chain. However, plant sterols differ in their side chain configuration with an extra methyl constituent on carbon 24 in campesterol (structure not shown), an extra ethyl group on carbon 24 in sitosterol, and an extra ethyl group on carbon 24 along with a double bond between carbons 22 and 23 in stigmasterol (Fig. 1). The alkylated side chains of plant sterols make them more effective at ordering phospholipids and, thus cells containing plant sterols have reduced membrane fluidity compared to those containing cholesterol. There are likely >20 different plant sterol species and >40 xenosterols our diets may expose us to [5].

The relative amounts of cholesterol and plant sterols consumed in the diet depend on their composition in animal fat and vegetable oils. A cholesterol:plant sterol ratio of ~1 is seen in a typical Western diet with daily intakes of approximately 250-400 mg of cholesterol and 200-400 mg of plant sterols [6-8]. Despite comparable amounts of ingested cholesterol and plant sterols, circulating plant sterol levels are less than 0.5 mg/dL as compared to cholesterol levels of ~42.5-208 mg/dL. Given that humans do not synthesize plant sterols (and have a very limited ability to metabolize them) [9], the substantially lower plasma concentrations of plant sterols is due primarily to a much lower absorption of plant sterols in humans that is approximately one-tenth that of cholesterol [10].

Although the majority of total body cholesterol is derived from *de novo* cholesterol synthesis [11], it is now evident that dietary absorption significantly contributes to maintaining total body sterol homeostasis [12]. All sterols need to be solubilized in micelles for absorption into enterocytes. Plant sterols effectively compete with, and displace, cholesterol from micelles and can thus decrease cholesterol absorption [10]. The influx of dietary cholesterol

regulates the its *de novo* synthesis. The liver is a key organ in maintaining this balance. Excess body cholesterol is eliminated exclusively by the liver, either by direct excretion as free cholesterol into bile or by its conversion into bile acids for excretion in the bile. The small amounts of dietary plant sterols allowed entry into the body are rapidly excreted by the liver into bile. Thus, humans are equipped with an efficient, discriminating mechanism to retain cholesterol and exclude xenosterols that may be deleterious to the body [13]. Research of the molecular defects leading to the disrupted sterol physiology seen in Sitosterolemia has identified the proteins involved in the selective absorption of cholesterol in the intestine and elimination of plant sterols, as well as excess cholesterol, by the liver.

Sitosterolemia

Sitosterolemia was first reported by Bhattacharyya and Connor in 1973 [2]. They identified two sisters with tendon xanthomas and elevated plasma concentrations of the plant sterols sitosterol, campesterol, and stigmasterol but non-significant elevations in plasma cholesterol. The disease was named β -sitosterolemia due to the finding that sitosterol was the most plentiful plant sterol. The ' β ' is not necessary as there is no natural ' α ' enantiomer of sitosterol. Further investigation would identify an autosomal recessive inheritance pattern [14] and a highly-developed mechanism important in dietary sterol trafficking [15-17]. More than 100 cases of the disease have since been reported world-wide, so it a genuine rare disorder [18]. There are more than 20 different known species of plants sterols along with other non-cholesterol sterols found in non-plant species such as shell-fish and these are all retained in subjects with Sitosterolemia [19]. The finding that all non-cholesterol sterols are effectively excluded by the human body highlights an extremely effective mechanism designed to prevent absorption of xenosterols. Discoveries in the study of Sitosterolemia have transformed our understanding of sterol-trafficking conducted by intestinal and hepatobiliary exporters. We now know that a significant amount of sterols are pumped out of the body by ABCG5 and ABCG8, and that xenosterols are preferentially eliminated along with excess cholesterol [20, 21].

Clinical Features and diagnosis

The clinical features that may be seen in Sitosterolemia include premature atherosclerosis, tendon xanthomas, arthralgia/arthritis, endocrine insufficiency, and liver dysfunction [3]. In contrast to adults who typically have mildly elevated total cholesterol, young children affected by the disease may present with markedly elevated plasma cholesterol levels [22]. Hematologic abnormalities, such as hemolysis, thrombocytopenia, and stomatocytosis, are frequently seen in affected individuals and are believed related to the high plasma levels of plant sterols [3]. Of significance, sitosterolemic patients exhibit accelerated aortic atherosclerosis and symptomatic coronary artery disease that can lead to life-threatening myocardial infarction in adolescence and young adulthood [23, 24]. Cases of death from acute myocardial infarction have been reported in children as young as 13 years, with autopsy findings revealing extensively atherosclerotic coronary arteries [23, 25]. There appears to be no sex difference in the predilection for premature obstructing atherosclerosis of the aorta or coronary and carotid vessels in sitosterolemic patients, and aortic valve involvement is not infrequent.

Diagnosis of the disease is confirmed by identifying elevated plasma and tissue levels of plant sterols. Normal humans have barely detectable plant sterol levels that are < 0.5 mg/dL. In contrast, individuals with Sitosterolemia have plant sterol levels greater than 10 mg/dL. Laboratory testing for the accumulation of plant sterols requires GC or HPLC techniques to distinguish between plant sterols and cholesterol. No other known disease has been associated with elevations in plasma plant sterol levels. In some situations, increased plant sterol levels have been seen in patients receiving intravenous soybean based lipid emulsions [26]. Post-mortem tissue accumulation can be seen in almost all tissues with the exception of the brain, demonstrating the important role of the blood-brain barrier in protecting the brain [23]. However, in animal models of sitosterolemia, plant sterol accumulation has been demonstrated [27-29], albeit at much lower levels; the clinical significance of this remains to be elucidated.

Genetics

Sitosterolemia is an autosomal recessive disease [17]. The disease was mapped to the *STSL* locus on human chromosome 2p21. The *STSL* locus is comprised of two highly homologous genes, *ABCG5* and *ABCG8*, arranged in a head-to-head organization [30-32]. Only 140 bp separate the initiation sites of the two genes, each gene is made up of 13 exons and 13 introns, are evolutionary conserved and a primordial gene duplication event is hypothesized to have led to the creation of two genes (Fig. 2). Since these genes have been identified from fish to man, their role in a conserved function of regulating whole-body sterol stores seems well-supported [33]. Although transcriptional factors such as LXR are known to regulate expression of these genes, the details of this regulation remain to be elucidated.

The proteins encoded, sterolin-1 (*ABCG5*) and sterolin-2 (*ABCG8*), belong to the ATP-binding cassette family G and act as obligate heterodimers. They act as mutual chaperones during protein synthesis to help attain a final mature stable structure; if one subunit is mutant, the expression of the other is affected, highlighting the obligatory nature [34-36]. Thus, mutations leading to two defective copies of *ABCG5* or two defective copies of *ABCG8* cause the Sitosterolemia. Parents of affected individuals are obligate carriers for one defective allele, but sufficient normal *ABCG5/ABCG8* function (theoretically 50% loss) results in a normal phenotype, although transient rises in plant sterols can be seen when fed margarines fortified with them [37]. The crucial feature of Sitosterolemia is the disturbance of normal pathways that prevent the accumulation of plant sterols and other xenosterols. Discovery of the molecular defect causing Sitosterolemia has identified the long sought-after mechanism that underlies sterol excretion into bile and elucidated the requirement of normally functioning *ABCG5* and *ABCG8* for sterol elimination from the body. The absence of functioning *ABCG5* or *ABCG8* leads to hyper-absorption of all no-cholesterol sterols in the intestines and failure to excrete sterols into bile in the liver.

New insights into the pathophysiology of dietary sterol trafficking

Prior to the investigation of this rare disease, the literature and mainstream textbooks either ignored or glossed over how dietary cholesterol was absorbed; the importance of bile salts, emulsification and micelle formation was well-recognized, but how this allowed for the sterols to enter the enterocyte was never elaborated. At the other end, the link between bile

acid, phospholipid and cholesterol excretion/secretion into bile was also well-studied, but the mechanism(s) of how cholesterol traversed the membrane and whether this was an active or passive movement was never established, despite the centrality of biliary excretion as a major means to loose whole body cholesterol.

Intestinal Dietary Sterol Trafficking—Once the physiology of the defect in Sitosterolemia came into better focus, at least the role of gene product(s) encoded by the *STSL* locus was hypothesized, at the very least, to be involved in preventing dietary xenosterols from staying in the body. Early studies of bile from affected subjects hinted that the defect may also affect biliary sterol secretion, but it was clear that clearance of xenosterols was greatly affected [15]. Once the genetic defect was elucidated (with a contemporaneous advance made from studies of rare cholestasis disorders), the picture of a triad of ABC transporters that excreted bile salts (ABCB11), cholesterol (ABCG5/ABCG8) and phospholipid (ABCC4) into bile became much clearer [38], and ultimately supported by studies involving knock-out mouse models. However, how cholesterol was absorbed, as opposed to xenosterols/cholesterol excreted via ABCB5/ABCG8 still remained to be explained. The discovery of a novel drug, Ezetimibe by Davis and his colleagues at Schering-Plough Inc., opened up an avenue; although the molecular target of Ezetimibe (Zetia®) was not known, one hypothesis was that ABCG5/ABCG8 could be its target. This led to a study design to test if the drug was as efficacious in subjects with Sitosterolemia at lowering cholesterol, as it was in normal subjects in phase III studies [39]. Remarkably, not only did the drug work, it lowered plant sterols, and led to an FDA-approved indication for treatment (probably the only ‘blockbuster’ drug approved for a rare indication!). Since the target of Ezetimibe remained elusive, efforts were re-doubled at Schering and in a remarkably innovative effort, using multiple pathways and creativity, they identified another novel protein, the Niemann-Pick C1-like 1 (NPC1L1) [40, 41]. In the space of 4y, how sterols are absorbed and excreted by the mammalian body to regulate whole-body sterol balance protein was established with *bona fide* molecular targets. Subsequent work has helped solidify these concepts and extended these to include the role of the intestine to also directly excrete cholesterol [42]. Current concepts now embody a scenario whereby dietary sterols are digested by pancreatic cholesterol esterase to yield free sterols, which then compete with each other to gain entry into micelles formed by mixing with biliary cholesterol, phospholipids and bile salts. Failure to form micelles will prevent all subsequent dietary sterol absorption and represents an obligatory step. Mediated by NPC1L1, the micelle content (sterols but seemingly not the bile salts) gain entry into the enterocyte. The exact molecular details of this process are also unclear, but may involve vesicular trafficking involving NPC1L1 [43-45]. Once inside the enterocyte, cholesterol is preferentially esterified via ACAT-2 [46], whereas non-cholesterol sterols remain primarily unesterified, and presumably are pumped back into the intestinal lumen via ABCG5/ABCG8 (See Fig. 3, left-hand panel). A small amount of free xenosterols do end up getting incorporated into chylomicrons (estimated to be <5% of dietary intake) and can be thus secreted into lymph [47]. It should be noted that intestinal ABCG5/ABCG8 may also be responsible for some net excretion of dietary cholesterol at the enterocyte level, thus diminishing intestinal absorption; in *Abcg8* knockout mice, studies involving thoracic duct cannulation who that

there is increased dietary cholesterol flux (presumably because there is less enterocyte excretion) [48].

Hepatic Dietary sterol trafficking—Chylomicrons, containing dietary lipids, are progressively metabolized in the capillary endothelial beds, which remove primarily triglycerides [49] and the sterol-enriched remnants are rapidly cleared by the liver. In the liver, dietary cholesterol can enter the metabolic pathway (re-esterification, re-secretion in VLDL, broken down to bile salts, or can be stored), but xenosterols are preferentially diverted to biliary secretion, via ABCG5/ABCG8 (see right-hand panel, Fig. 3). The net resultant effect is an average daily dietary cholesterol absorption of 55%, but <1% xenosterol absorption [50]. Animal models to mimic sitosterolemia have now been reported and have expanded our understanding of hepatic dietary sterol metabolism. A phenotype in mice that resemble human disease is seen when both *ABCG5* and *ABCG8* are disrupted [27]. *ABCG5/ABCG8* knockout mice demonstrate sterol hyper-absorption and the inability to excrete sterols into bile. Mice with over-expression of human ABCG5 and ABCG8 exhibit super saturation of cholesterol in bile, excretion of large quantities of neutral sterols, reduced plasma plant sterol levels, and a compensatory up-regulation of the cholesterol synthesis rates [51]. These animals also showed lower cholesterol absorption rates and lower plasma HDL cholesterol levels. In addition, over-expression of hepatic ABCG5 and ABCG8 is associated with reduced production of apoB-lipoproteins and atherosclerosis. The liver is also the center of integration of whole-body cholesterol trafficking [52]. Excess whole-body cholesterol, from synthesis in the periphery is sent back to the liver, where it can be directly excreted into bile via ABCG5/ABCG8, or converted into bile salts and excreted into bile via ABCB12. In humans, but not rodents, NPC1L1 is also expressed in the liver and presumably is responsible for re-uptake of biliary cholesterol (perhaps evolved to retain cholesterol in the body). The reader is directed to a recent review of this subject [53].

Xenosterols and the Hematopoietic system—Erythrocytes were known to be able to take up and partition plant sterols into their membranes and hemolysis was a known associated feature of Sitosterolemia (together with splenomegaly). However, much more focus and attention was drawn to this aspect by Rees *et al* who showed that mutations at the *STSL* locus were responsible for a clinical condition known as Mediterranean stomatocytosis/macrophage thrombocytopenia [54]: affected subjects had macrothrombocytopenia. Interestingly, dietary plant oils were shown to be toxic to a well-known in-bred rat line [55], the spontaneously hypertensive rat-stroke prone (SHR-SP), and plant sterols affected the rate of hemolysis of blood isolated from these animals [56]. Both SHR and SHR-SP lines are mutant for *Abcg5* and were sitosterolemic when raised on standard rodent chow [57, 58]. Thus plant sterols seem to lead to increased red cell fragility by partitioning preferentially in the outer leaflet of the red blood cell membrane, though the effects on platelets physiology is more complex. Artificially raising plant sterols, as in children receiving total parental nutrition, elevated plant sterols were associated with cholestasis (see below) and thrombocytopenia [26]. Macrothrombocytopenia was further recapitulated in two mouse models; in the *Abcg5* knockout mouse line [59] and a naturally-occurring mutation affecting *Abcg5* in the *trac* mouse line [60]. In both of these reports, the effect of phytosterolemia on the megakaryocyte membrane demarcation was felt to be responsible for the phenotype of

macrothrombocytopenia, with the *trac* mouse also exhibiting cardiomyopathy and leukopenia. In both cases, the platelet phenotypes could be rescued by lowering plant sterols, in the case of the *Abcg5* knockout mice by the use of ezetimibe [59], and in the *trac* mouse by using transgenic complementation [60]. Thus plant sterols affect megakaryocytes at the later stages of maturation and in a reversible manner. More recently, phytosterolemia was shown hyperactivate platelets, with constitutive binding of fibrinogen to its $\alpha\text{IIb}\beta 3$ integrin receptor and internalization, increased generation of platelet-derived microparticles, and changes in the quantity and subcellular localization of filamin [61]. What is not clear is how plant sterols effect these changes; is it by altering cholesterol levels in the membranes, or is it via specific plant sterol species? It is also not clear if there is a particular plant sterol level that needs to be attained to bring about these toxic changes.

Xenosterols and other organ dysfunction—We reported a case of ‘idiopathic liver cirrhosis’ who was diagnosed because of screening of clinical samples who had elevated plant sterols, and DNA analyses showed he had mutations in *ABCG8* [62]. Liver transplantation significantly ameliorated his phytosterol levels [62]. Traditional total parental nutrition (TPN) formulae contain significant amounts of phytosterols and while adult use of TPN is rarely associated with liver dysfunction, hemolysis or thrombocytopenia, pediatric use of TPN, especially prolonged use has been reported to cause these conditions [26]. It is not clear if the phytosterols are directly causative, whether these changes are an epiphenomenon or whether the liver damage is multi-factorial with xenosterols as one potential factor.

Case reports indicate that phytosterols can also affect endocrine function [63], supported by *in vitro* data that the adrenal gland may have decreased function when exposed to plant sterols [64]. Although humans with sitosterolemia are not noted to be infertile, mice with mutations of *Abcg5* or *Abcg8* exhibit significant infertility, despite normal endocrine organ development [60, 65]. Although these mice also exhibit severely decreased life-spans [60, 65, 66], the cause of death is not clear. Histological changes in cardiac structure have been documented [60, 66], though whether this translates to cardiac dysfunction has not been demonstrated.

Additionally, a profound loss of fat stores in *Abcg8* knockout mice has been reported [65]. Infertility, as well as fat loss can be mitigated by blocking dietary sterol entry using ezetimibe or diets low in plant sterols, suggesting all of these toxic effects are reversible [65].

Conclusions

The molecular mechanisms that regulate whole body sterol homeostasis are essential for maintenance of normal health. Investigations of sitosterolemia, a rare, autosomal recessive sterol storage disorder, have led to the identification of two key molecules, *ABCG5* and *ABCG8* (sterolins), critical in sterol-regulating pathways. The loss of these proteins allows the accumulation of all of the dietary sterols, but more importantly xenosterols. Clinical, genomic, and animal studies of this disease have greatly increased our understanding of physiological processes involved in regulating dietary sterol absorption and excretion. When

this process is defective, xenosterols accumulate (as does cholesterol) and leads to premature atherosclerosis, macrothrombocytopenia, and rarely liver and endocrine dysfunction, highlighting the key concept that we need to regulate sterol pools, and defend against xenosterol accumulation.

Many questions remain regarding how xenosterols lead to pathophysiology; it is not clear if this is a direct toxic effect of one or more xenosterol species, or whether the pathophysiology is an indirect effect by altering cholesterol trafficking. It is also not clear if low level exposure has any cumulative effect on pathophysiology; we are exposed via the diet to xenosterols on a daily basis and, despite normal sterolin function, there is some variation in xenosterols in the plasma. What is clear is that these sterol-regulating proteins have established a key link between diet, genetics, and environment, and the hope is that continued research will bring us closer to uncovering the molecular basis for the connection between diet, atherosclerosis and heart disease.

References

- 1*. Schoenheimer R. Über die Bedeutung der Pflanzensterine für den tierischen Organismus. Hoppe-Seyler's Z für physiol Chem. 1929; 180:1–5. A classic paper that outline the key issues almost a century ago.
- 2**. Bhattacharyya AK, Connor WE. Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J Clin Invest. 1974; 53(4):1033–1043. One of best examples of how to investigate a rare human disorder; the authors predicted the pathological basis well before the genetic defect was identified. [PubMed: 4360855]
3. Patel, SB., Salen, G. Sitosterolemia: xenophobia for the body. In: Vissers, MN, Kastelein, JJP., Stroes, ES., editors. Evidence-based Management of Lipid Disorders. tfm Publishing Ltd; Harley: 2010. p. 217–230.
4. Mouritsen OG, Zuckermann MJ. What's so special about cholesterol? Lipids. 2004; 39(11):1101–1113. [PubMed: 15726825]
5. Weihrauch JL, Gardner JM. Sterol content of foods of plant origin. J Am Diet Assoc. 1978; 73(1): 39–47. [PubMed: 659760]
6. Nair PP, Turjman N, Kessie G, et al. Diet, nutrition intake, and metabolism in populations at high and low risk for colon cancer. Dietary cholesterol, beta-sitosterol, and stigmasterol. Am J Clin Nutr. 1984; 40(4 Suppl):927–930. [PubMed: 6486101]
7. Normen AL, Brants HA, Voorrips LE, Andersson HA, van den Brandt PA, Goldbohm RA. Plant sterol intakes and colorectal cancer risk in the Netherlands Cohort Study on Diet and Cancer. Am J Clin Nutr. 2001; 74(1):141–148. [PubMed: 11451730]
8. Valsta LM, Lemstrom A, Ovaskainen ML, et al. Estimation of plant sterol and cholesterol intake in Finland: quality of new values and their effect on intake. Br J Nutr. 2004; 92(4):671–678. [PubMed: 15522137]
9. Salen G, Ahrens E Jr, Grundy SM. Metabolism of beta-sitosterol in man. J Clin Invest. 1970; 49(5): 952–967. [PubMed: 5441548]
10. Gould RG, Jones RJ, LeRoy GV, Wissler RW, Taylor CB. Absorbability of beta-sitosterol in humans. Metab. 1969; 18(8):652–662.
11. Jolley CD, Woollett LA, Turley SD, Dietschy JM. Centripetal cholesterol flux to the liver is dictated by events in the peripheral organs and not by the plasma high density lipoprotein or apolipoprotein A-I concentration. J Lipid Res. 1998; 39(11):2143–2149. [PubMed: 9799800]
12. McMurry MP, Connor WE, Cerqueira MT. Dietary cholesterol and the plasma lipids and lipoproteins in the Tarahumara Indians: a people habituated to a low cholesterol diet after weaning. Am J Clin Nutr. 1982; 35(4):741–744. [PubMed: 7200320]

13. Bhattacharyya AK, Connor WE, Spector AA. Excretion of sterols from the skin of normal and hypercholesterolemic humans. Implications for sterol balance studies. *J Clin Invest.* 1972; 51(8): 2060–2070. [PubMed: 5054464]
14. Beaty TH, Kwiterovich P Jr, Khoury MJ, et al. Genetic analysis of plasma sitosterol, apoprotein B, and lipoproteins in a large Amish pedigree with sitosterolemia. *Am J Hum Genet.* 1986; 38(4): 492–504. [PubMed: 3706300]
15. Salen G, Shore V, Tint GS, et al. Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. *J Lipid Res.* 1989; 30(9):1319–1330. [PubMed: 2600539]
16. Salen G, Tint GS, Shefer S, Shore V, Nguyen L. Increased sitosterol absorption is offset by rapid elimination to prevent accumulation in heterozygotes with sitosterolemia. *Arterioscler Thromb.* 1992; 12(5):563–568. [PubMed: 1576118]
17. Patel SB, Salen G, Hidaka H, et al. Mapping a gene involved in regulating dietary cholesterol absorption. The sitosterolemia locus is found at chromosome 2p21. *J Clin Invest.* 1998; 102(5): 1041–1044. [PubMed: 9727073]
18. Lu K, Lee MH, Hazard S, et al. Two genes that map to the STSL locus cause sitosterolemia: Genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8 respectively. *Am J Hum Genet.* 2001; 69:278–290. [PubMed: 11452359]
- 19*. Gregg RE, Connor WE, Lin DS, Brewer H Jr. Abnormal metabolism of shellfish sterols in a patient with sitosterolemia and xanthomatosis. *J Clin Invest.* 1986; 77(6):1864–1872. Not just plant sterols, but even shellfish sterols can accumulate in sitosterolemia. [PubMed: 3711338]
20. Kidambi S, Patel SB. Sitosterolaemia: pathophysiology, clinical presentation and laboratory diagnosis. *J Clin Pathol.* 2008; 61(5):588–594. [PubMed: 18441155]
21. Yu L, von Bergmann K, Lutjohann D, Hobbs HH, Cohen JC. Selective sterol accumulation in ABCG5/ABCG8-deficient mice. *J Lipid Res.* 2004; 45(2):301–307. [PubMed: 14657202]
22. Lee MH, Lu K, Patel SB. Genetic basis of sitosterolemia. *Curr Opin Lipidol.* 2001; 12(2):141–149. [PubMed: 11264985]
23. Salen G, Horak I, Rothkopf M, et al. Lethal atherosclerosis associated with abnormal plasma and tissue sterol composition in sitosterolemia with xanthomatosis. *J Lipid Res.* 1985; 26(9):1126–1133. [PubMed: 4067433]
24. Mymin D, Wang J, Frohlich J, Hegele RA. Image in cardiovascular medicine. Aortic xanthomatosis with coronary ostial occlusion in a child homozygous for a nonsense mutation in ABCG8. *Circulation.* 2003; 107(5):791. [PubMed: 12578886]
25. Kolovou G, Voudris V, Drogari E, Palatianos G, Cokkinos DV. Coronary bypass grafts in a young girl with sitosterolemia. *Eur Heart J.* 1996; 17(6):965–966. [PubMed: 8781841]
26. Clayton PT, Bowron A, Mills KA, Massoud A, Casteels M, Milla PJ. Phytosterolemia in children with parenteral nutrition-associated cholestatic liver disease. *Gastroenterology.* 1993; 105(6): 1806–1813. [PubMed: 8253356]
27. Yu L, Hammer RE, Li-Hawkins J, et al. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc Natl Acad Sci U S A.* 2002; 99(25):16237–16242. [PubMed: 12444248]
28. Vanmierlo T, Weingartner O, van der Pol S, et al. Dietary intake of plant sterols stably increases plant sterol levels in the murine brain. *J Lipid Res.* 2012; 53(4):726–735. [PubMed: 22279184]
29. Vanmierlo T, Rutten K, van Vark-van der Zee LC, et al. Cerebral accumulation of dietary derivable plant sterols does not interfere with memory and anxiety related behavior in Abcg5^{-/-} mice. *Plant Foods Hum Nutr.* 2011; 66(2):149–156. [PubMed: 21431910]
30. Lee MH, Lu K, Hazard S, et al. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat Gen.* 2001; 27:79–83.
31. Lee MH, Hazard S, Carpten JD, et al. Fine-mapping, mutation analyses, and structural mapping of cerebrotendinous xanthomatosis in U.S. pedigrees. *J Lipid Res.* 2001; 42(2):159–169. [PubMed: 11181744]
32. Berge KE, Tian H, Graf GA, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science.* 2000; 290(5497):1771–1775. [PubMed: 11099417]

33. Hazard SE, Patel SB. Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. *Pflugers Arch.* 2007; 453(5):745–752. [PubMed: 16440216]
34. Graf GA, Li WP, Gerard RD, et al. Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. *J Clin Invest.* 2002; 110(5):659–669. [PubMed: 12208867]
35. Graf GA, Yu L, Li WP, et al. ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. *J Biol Chem.* 2003; 278(48):48275–48282. [PubMed: 14504269]
36. Graf GA, Cohen JC, Hobbs HH. Missense mutations in ABCG5 and ABCG8 disrupt heterodimerization and trafficking. *J Biol Chem.* 2004; 279(23):24881–24888. [PubMed: 15054092]
37. Kwiterovich PO Jr, Chen SC, Virgil DG, Schweitzer A, Arnold DR, Kratz LE. Response of obligate heterozygotes for phytosterolemia to a low-fat diet and to a plant sterol ester dietary challenge. *J Lipid Res.* 2003; 44(6):1143–1155. [PubMed: 12671028]
38. Klaassen CD, Aleksunes LM. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev.* 2010; 62(1):1–96. [PubMed: 20103563]
39. Salen G, von Bergmann K, Lutjohann D, et al. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation.* 2004; 109(8):966–971. [PubMed: 14769702]
40. Altmann SW, Davis HR Jr, Zhu LJ, et al. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science.* 2004; 303(5661):1201–1204. [PubMed: 14976318]
41. Davis HR Jr, Zhu LJ, Hoos LM, et al. Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *J Biol Chem.* 2004; 279(32):33586–33592. [PubMed: 15173162]
42. van der Velde AE, Brufau G, Groen AK. Transintestinal cholesterol efflux. *Curr Opin Lipidol.* 2010; 21(3):167–171. [PubMed: 20410820]
43. Ge L, Wang J, Qi W, et al. The cholesterol absorption inhibitor ezetimibe acts by blocking the sterol-induced internalization of NPC1L1. *Cell Metab.* 2008; 7(6):508–519. [PubMed: 18522832]
44. Xie C, Zhou ZS, Li N, et al. Ezetimibe blocks the internalization of NPC1L1 and cholesterol in mouse small intestine. *J Lipid Res.* 2012; 53(10):2092–2101. [PubMed: 22811412]
45. Xie C, Li N, Chen ZJ, Li BL, Song BL. The small GTPase Cdc42 interacts with Niemann-Pick C1-like 1 (NPC1L1) and controls its movement from endocytic recycling compartment to plasma membrane in a cholesterol-dependent manner. *J Biol Chem.* 2011; 286(41):35933–35942. [PubMed: 21844200]
46. Temel RE, Gebre AK, Parks JS, Rudel LL. Compared with Acyl-CoA:Cholesterol O-Acyltransferase (ACAT) 1 and Lecithin:Cholesterol Acyltransferase, ACAT2 Displays the Greatest Capacity to Differentiate Cholesterol from Sitosterol. *J Biol Chem.* 2003; 278(48):47594–47601. [PubMed: 12975367]
47. Kuksis A, Huang TC. Differential absorption of plant sterols in the dog. *Can J Biochem Physiol.* 1962; 40:1493–1504.
48. Wang HH, Patel SB, Carey MC, Wang DQ. Quantifying anomalous intestinal sterol uptake, lymphatic transport, and biliary secretion in *Abcg8*(^{-/-}) mice. *Hepatology.* 2007; 45(4):998–1006. [PubMed: 17393508]
49. Young SG, Zechner R. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. *Genes Dev.* 2013; 27(5):459–484. [PubMed: 23475957]
50. Bosner MS, Lange LG, Stenson WF, Ostlund RE Jr. Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. *J Lipid Res.* 1999; 40(2):302–308. [PubMed: 9925660]
51. Yu L, Li-Hawkins J, Hammer RE, et al. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J Clin Invest.* 2002; 110(5):671–680. [PubMed: 12208868]
52. Dietschy JM, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J Lipid Res.* 1993; 34(10):1637–1659. [PubMed: 8245716]
53. Pramfalk C, Jiang ZY, Parini P. Hepatic Niemann-Pick C1-like 1. *Curr Opin Lipidol.* 2011; 22(3):225–230. [PubMed: 21494140]

54. Rees DC, Iolascon A, Carella M, et al. Stomatocytic haemolysis and macrothrombocytopenia (Mediterranean stomatocytosis/macrothrombocytopenia) is the haematological presentation of phytosterolaemia. *Br J Haematol.* 2005; 130(2):297–309. [PubMed: 16029460]
55. Huang MZ, Naito Y, Watanabe S, et al. Effect of rapeseed and dietary oils on the mean survival time of stroke-prone spontaneously hypertensive rats. *Biol Pharm Bull.* 1996; 19(4):554–557. [PubMed: 8860957]
56. Ratnayake WM, L'Abbe MR, Mueller R, et al. Vegetable oils high in phytosterols make erythrocytes less deformable and shorten the life span of stroke-prone spontaneously hypertensive rats. *J Nutr.* 2000; 130(5):1166–1178. [PubMed: 10801914]
57. Scoggan KA, Gruber H, Lariviere K. A missense mutation in the *Abcg5* gene causes phytosterolemia in SHR, stroke-prone SHR, and WKY rats. *J Lipid Res.* 2003; 44(5):911–916. [PubMed: 12611906]
58. Yu H, Pandit B, Klett E, et al. The rat *STSL* locus: characterization, chromosomal assignment, and genetic variations in sitosterolemic hypertensive rats. *BMC Cardiovasc Disord.* 2003; 3(1):4. [PubMed: 12783625]
59. Kruit JK, Drayer AL, Bloks VW, et al. Plant sterols cause macrothrombocytopenia in a mouse model of sitosterolemia. *J Biol Chem.* 2008; 283(10):6281–6287. [PubMed: 18156627]
60. Chase TH, Lyons BL, Bronson RT, et al. The mouse mutation “thrombocytopenia and cardiomyopathy” (*trac*) disrupts *Abcg5*: a spontaneous single gene model for human hereditary phytosterolemia/sitosterolemia. *Blood.* 2010; 115(6):1267–1276. [PubMed: 19846887]
61. Kanaji T, Kanaji S, Montgomery RR, Patel SB, Newman PJ. Platelet hyperreactivity explains the bleeding abnormality and macrothrombocytopenia in a murine model of Sitosterolemia. *Blood.* 2013 In Press.
62. Miettinen TA, Klett EL, Gylling H, Isoniemi H, Patel SB. Liver transplantation in a patient with sitosterolemia and cirrhosis. *Gastroenterology.* 2006; 130(2):542–547. [PubMed: 16472606]
63. Mushtaq T, Wales JK, Wright NP. Adrenal insufficiency in phytosterolaemia. *Eur J Endocrinol.* 2007; 157(Suppl 1):S61–65. [PubMed: 17785700]
64. Yang C, Yu L, Li W, Xu F, Cohen JC, Hobbs HH. Disruption of cholesterol homeostasis by plant sterols. *J Clin Invest.* 2004; 114(6):813–822. [PubMed: 15372105]
65. Solca C, Tint GS, Patel SB. Dietary xenosterols lead to infertility and loss of abdominal adipose tissue in sterolin-deficient mice. *J Lipid Res.* 2013; 54(2):397–409. [PubMed: 23180829]
66. McDaniel AL, Alger HM, Sawyer JK, et al. Phytosterol feeding causes toxicity in *ABCG5/G8* knockout mice. *Am J Pathol.* 2013; 182(4):1131–1138. [PubMed: 23380580]
67. Lu K, Lee M, Patel SB. Dietary cholesterol absorption; more than just bile. *Trends Endo Metab.* 2001; 12(7):314–320.

Executive summary

Sitosterolemia

- Autosomal recessive rare disorder
- Mutation in ABCG5 or ABCG8 are causative
- Diagnostically elevated plant sterols in blood
- Classically associated with tendon xanthomas and premature atherosclerosis

New Insights

- Xenosterol accumulation affects all organ systems
- Pure Macrothrombocytopenia as only presenting feature
- Can cause liver dysfunction
- Xenosterol can accumulate in the CNS
- May also act as endocrine disruptors

Figure 1.

A representative set of sterol structures.

The first structure shown is cholesterol, and all other structures are sterols that are xenosterols.

The latter are not synthesized in the human body and most are also considered to be toxic, if they accumulate in the body. The structure of campesterol is not shown.

STSL Locus Chromosome 2p21

Figure 2.

Gene structure of the *STSL* locus.

The Sitosterolemia locus, *STSL*, comprises of two highly homologous genes, *ABCG5* and *ABCG8*, that are thought to have arisen by a gene duplication even very early in evolution as this organization is present and conserved from fish to man. There is no classical promoter, the genes being transcribed in an opposite direction and are separated by no more than 140 bp of sequence. Figure adapted from reference [22].

Figure 3.

Sterol trafficking pathways.

The process of absorption of dietary sterol absorption begins with secretion of bile containing bile salts, free cholesterol and phospholipids that then mix with dietary sterols to form micelles. These interact with NPC1L1, allowing sterol entry into the enterocyte. ACAT-2 esterifies cholesterol allowing the bulk of this to proceed into chylomicrons and any plant sterols absorbed remain unesterified and are excreted by ABCG5/ABCG8 into the lumen. However, a small amount of plant sterols can make it into chylomicrons. In the blood, chylomicrons are metabolized with progressive loss of triglycerides, relative enrichment of sterols and these remnants are cleared by the liver. Any excess cholesterol, as well as any of the xenosterols are excreted by the liver ABCG5/ABCG8 into bile for elimination. ApoB; apolipoprotein B, ApoE; apolipoprotein E, ACAT-2; Acyl-CoA:cholesterol acyl-transferase, BSEP; bile salt export protein (also ABCB12), HDL: high density lipoproteins, LDL-R; low density lipoprotein receptor, MDR2/3; Multiple drug resistance protein (also ABCB4), MTP; microsomal triglyceride transfer protein, Ntcp; sodium/taurocholate co-transporting polypeptide, PL; phospholipid, SR-B1: Scavenger receptor B1. Figure adapted from reference [67].