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Plant Sterols and Stanols: Their Role in Health and Disease

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Abstract

Mammalian physiological processes, and likely any organism with a biliary tree, can distinguish between dietary cholesterol and non-cholesterols, retaining very little of the non-cholesterol in their bodies. Historically, the distinction between plant sterols and cholesterol has been known about for a century or more. That plants sterols were not 'absorbed' has been investigated for almost half a century. Indeed, the oral of plant sterols in gram quantities was shown to interfere with cholesterol absorption and is one of the oldest pharmacological therapies for hypercholesterolemia. Although the basis for the latter was shown to be caused by exclusion of cholesterol from intestinal micelles by plant sterols, it was not until the identification of the a rare genetic disease, sitosterolemia, first described in 1974, that led to the hypothesis that specific molecular mechanism(s) governed both the entry and excretion of sterols by the body. This talk will cover the physiology of dietary sterol metabolism, genetics and pathophysiology of sitosterolemia. Additionally, the role of plant sterols in normal and abnormal metabolism in humans as well as selected animal models will be discussed.

Introduction

Dietary cholesterol absorption from the intestine is a critical component of cholesterol homeostasis. Under normal circumstances, the human diet contains approximately equal amounts of cholesterol and plant sterols. However, normal individuals retain on average approximately 50% of dietary cholesterol, but less than 5% of dietary plant sterols[1]. The normal physiologic process for handling dietary sterols by the gastrointestinal tract involves processing 200 to 500 mg of dietary cholesterol, but also 200 to 400 mg of phytosterols that are a component of plant sterols (Fig. 1). Almost all of the dietary phytosterols undergo fecal excretion along with 800 to 1500 mg of cholesterol plus its metabolic product, bile acids. The normal body is able to discriminate between cholesterol and non-cholesterol sterols[2]. This suggests that a molecular mechanism exists in the body that specifically retains cholesterol over non-cholesterol sterols [3]. The exact molecular mechanisms by which preferential cholesterol absorption occurs, while non-cholesterol sterols are not absorbed has not been fully elucidated, but clues from a rare genetic disorder, as well as a novel drug have led to a major insights.

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There are no significant disclosures.

The purpose of this review is to provide and overview of plant sterols and their role in health and disease. Included will be a discussion of the genetics of sitosterolemia, the role of plant sterols as markers for premature atherosclerosis, and potential treatment options for lowering plant sterol levels in the body.

Metabolism of Cholesterol and Plant Sterols

The enterohepatic metabolism of cholesterol and plant sterols is complex (Fig. 2). The metabolic process occurs within the intestinal lumen, where dietary cholesterol (and plant sterols) is reduced to free sterols by esterases, and transferred into micelles (a mixture of bile salts, phospholipids, free sterols and some fatty acids). These micelles interact with the enterocyte apical membrane (a process that has not been characterized at the molecular level) and allow entry of the sterols into the enterocyte. Cholesterol enters the metabolic pool, where it is esterified by ACAT-2[4], and incorporated into chylomicrons that are secreted at the basolateral surface into the lacteals that eventually drain into the venous circulation. Chylomicrons are acted upon by lipoprotein lipase in the capillary beds of all the organs, which allows for the dietary triglycerides as well as some fat-soluble vitamins to be delivered to these tissues. Sterols in these particles are not transferred out and remain as part of the remnant particles which are now recognized by receptors on the liver and cleared. Hence the bulk of the dietary sterols are delivered to the liver. Cholesterol enters the metabolic pool and can be re-packaged into VLDL and secreted back into the circulation, whereas non-cholesterol sterols are excreted into the biliary tree and thus back into the lumen of the intestine.

How much dietary cholesterol do we absorb? The average person absorbs about 55% of normal dietary intake with a typical Gaussian distribution of rates; a small proportion of the population hyperabsorbs 90% of dietary cholesterol or underabsorbs less than 10%[5]. However, the dominant factor determining dietary cholesterol absorption for modern man is dietary intake rather than absorption efficiency. In contrast, intestinal absorption of plant sterols was thought to be negligible, although these different sterols are physically very similar (Fig. 3). The molecular mechanism underlying the exclusion of non-cholesterol sterols (xenosterols) was not considered until the discovery of a rare genetic disorder, sitosterolemia.

Sitosterolemia

A 22-year-old woman presented at the orthopedic clinic complaining of pain in both heels and knees. She had tendon xanthomas with a history of hand xanthomas beginning at age 8 years, which progressed to patellar, plantar, and Achilles tendon involvement. The patient has a sister who has a similar phenotype. This combination of arthralgia and tendon xanthomas is associated with the diagnosis of familial hypercholesterolemia. Familial hypercholesterolemia is characterized by hypercholesterolemia, tendon xanthoma, arthralgias and a family history of premature heart disease. It is relatively common and occurs in 1 in 200 to 500 of the general population. Diagnosis is typically confirmed by a lipid panel, which reveals an LDL-cholesterol level >200 mg/dL accompanied by a positive family history of premature heart disease and hypercholesterolemia. In the two sisters, the total cholesterol levels were borderline high at 195 mg/dL and 206 mg/dL. Serum triglyceride levels were 81 mg/dL and 63 mg/dL. Their parents had tendon xanthomas, but were otherwise normal with no family history of hyperlipidemia or cardiovascular disease for the previous two generations. The absence of a family history is not consistent with a diagnosis of familial hypercholesterolemia.

The original description of the rare disorder β-sitosterolemia and xanthomatosis was reported by Bhattacharyya and Connor in 1974[6]. Sitosterolemia or phytosterolemia is an autosomal recessive disorder[7]. It is an exceedingly rare disorder that affects fewer than 1 patient per 1 million population, with about 20 cases reported so far in the US (though under-diagnosis is likely to be a factor). The underlying pathophysiology is increased absorption of dietary sterols,

loss of discrimination between cholesterol and non-cholesterol sterols and reduced excretion of sterols (cholesterol or non-cholesterol sterols) into bile[8, 9]. Sitosterolemia is associated with tendon xanthomas, increased coronary atherosclerosis, and hemolysis[7]. Included in the differential diagnosis is cerebrotendinous xanthomatosis. Laboratory testing reveals elevated plasma levels of phytosterols that are typically >10mg/dL, of which sitosterol is the major species.

Segregation analyses in a larger Amish family showed that sitosterolemia was inherited as an autosomal recessive disease, suggesting a single locus was defective[10]. This locus is thus responsible for determining dietary sterol discrimination, cholesterol absorption, and biliary cholesterol (sterol) secretion. In healthy individuals, mechanisms exist that permit the body to distinguish between sterols. In sitosterolemia, either absorption of sterols is increased or excretion is decreased resulting in markedly elevated plasma non-cholesterol levels. A major consequence of sitosterolemia is increased risk of cardiovascular disease [11]. Although the prevalence of premature atherosclerotic disease in patients with sitosterolemia has not been fully defined because of the rarity of the condition, it is estimated that more than half of patients have documented coronary artery disease (CAD). A greater understanding of the mechanisms involved in the metabolism of cholesterol might result in better treatment options for sitosterolemia, but also could provide a better understanding of cholesterol absorption and excretion.

Plasma Sterol Profiles In Sitosterolemia

Early work was undertaken to elucidate the mechanisms of sitosterolemia in individuals and families affected by the disorder[3, 12]. Plasma sitosterol levels, measured by gas chromatography (GC) or high pressure liquid chromatography (HPLC) in affected individuals, their obligate heterozygous parents, unaffected siblings, and normal controls were compared[13]. Most unaffected individuals had plasma sitosterol levels <1 mg/dL (Fig. 4). Sitosterol levels >1 mg/dL in three parents and three siblings may have reflected the use of HPLC rather than GC in these six subjects. All individuals with sitosterolemia had plasma sitosterol values >8 mg/dL (some of these were on treatment). When plasma cholesterol levels were examined in the same groups, four subjects (all children) in the affected cohort had markedly elevated plasma cholesterol. All four of these subjects had been classified as pseudo-homozygous familial hypercholesterolemia (as all had responded to ‘dietary’ therapy), but in actuality all four subjects were sitosterolemic. Although a few rare cases of ‘pseudo-homozygous FH have been reported that do not have sitosterolemia, all such pediatric cases should be screened for this disease, especially as a therapy is now available (see below).

Genetics of Sitosterolemia

We performed linkage analyses of 10 well-characterized pedigrees and mapped the sitosterolemia locus, *STSL*, to human chromosome 2p21, between microsatellite markers *D2S1788* and *D2S1352*[3, 12, 14]. The disease locus interval was narrowed further and a physical map of this region constructed that allowed for the positional cloning of the sitosterolemia ‘gene’[14].

The surprise was that two highly homologous genes, ABCG5 and ABCG8, comprised the *STSL* locus and mutations in ether were responsible for the disease[15-17].

ABCG5 and ABCG8 are ATP-Binding Cassette containing proteins related to the ‘G’ family, members of which contain the White proteins in *Drosophila*, involved in retinal pigment metabolism[18]. Each gene consists of 13 exons and encodes for a cytoplasmic N-terminal segment that contains the characteristic ABC signature motifs that can bind ATP, and a C-terminal segment that contains six putative membrane-spanning domains. The protein products

of these genes were named sterolin-1 and sterolin-2[15, 18]. Mutational analyses showed that both *ABCG5* and *ABCG8* are mutated in sitosterolemia. Interestingly, patients were either completely mutated for both copies of ABCG8 or both copies of ABCG5, but not a mutation of one in ABCG5 and one in ABCG8[18]. This finding indicates that the two proteins probably work as obligate heterodimers to provide the same function. Work from the Hobbs' laboratory has provided key data to support both the heterodimerization, as well as showing that coexpression is necessary for normal maturation of these proteins within the cell[19-23].

Based on this research, two models were proposed as to how sterolins function (Fig. 5). Model A proposed that the heterodimer functions to pump sterols out of the cell[13]. Compared with cholesterol, the sterolin complex exhibits a greater affinity for pumping sitosterol (xenosterols) out of the cell (enterocyte or hepatocyte), and the sterolin complex can pump cholesterol out in the absence of sitosterol. The pumping mechanism is likely to be limited by the availability of substrate, thus, heterozygous individuals would be predicted to be normal. Theoretically, the heterodimer complex of sterolin-1 and sterolin-2 could have a dual function - pumping cholesterol into the cells and pumping non-cholesterol sterols out of cells. This model is not supported by current data and model A is the favored mechanism.

Pathophysiology of Sitosterolemia

Once the gene defects were identified, it became possible to dissect the pathophysiology, using *in vitro* as well as animal models methodology. Absence of ABCG5, ABCG8 or both results in an elevation of plant sterols in the tissues and blood and a failure to excrete sterols into bile[24-31]. Over expression of ABCG5 and ABCG8 in mice transgenic for the human genes led to a super saturation of cholesterol in bile and protected animals against atherosclerosis. Careful studies in ABCG8 knockout mice showed that there was increased intestinal absorption of both cholesterol and non-cholesterol sterols. Together with a failure to excrete cholesterol, even when forced, loss of sterolins showed that these proteins are the cholesterol and non-cholesterol transporters for excretion at the intestinal and biliary level. Interestingly, mice heterozygous for ABCG8 loss showed an intermediate phenotype for biliary sterol excretion.

To investigate whether humans who are heterozygous for mutations in ABCG8 (first degree relatives of patients with sitosterolemia), 12 heterozygotes were fed diets low in fat and cholesterol or enriched with plant sterols or stanols[32]. Blood levels of plant sterols increased significantly in the heterozygotes while on the enriched diet, and LDL-cholesterol levels decreased significantly while on the low fat diet. These studies confirmed earlier smaller studies by Salen and colleagues suggesting that heterozygous individuals may have a subtle phenotype[33].

Are There Other Clinical Features That May Indicate Sitosterolemia?

A case was reported of a 19-year-old male who presented with chronic active liver disease and after an extensive evaluation was found to have sitosterolemia[34]. Liver disease has not been reported to be feature in sitosterolemia. In contrast, the diagnosis of ‘idiopathic cirrhosis’ is not an uncommon diagnosis in people presenting with progressive liver failure. Since sitosterolemia requires analyses of blood by GC or HPLC for these sterols, it is possible that such cases are not diagnosed. Interestingly, our patient underwent orthotopic liver transplantation, which reduced his plant sterols to near-normal values, suggesting that restoration of normal sterolin activity in the liver alone is sufficient to effect a cure[34].

Rees and colleagues reported five pedigrees with Mediterranean stomatocytosis or Mediterranean macrothrombocytopenia, which combines stomatocytic hemolysis with the presence of very large platelets[35]. All patients had grossly elevated plasma levels of phytosterols and all showed mutations in the ABCG5 and ABCG8 transporters. The authors

concluded that Mediterranean stomatocytosis/macrothrombocytopenia is caused by an excess of phytosterols, that phytosterolemia can be diagnosed based on the distinctive hematology findings including unexplained hemolysis and macrothrombocytopenia, and that phytosterolemia should be considered in the differential diagnosis of all patients with large platelets[35]. A subsequent follow up study of one of these families showed that sitosterolemia was also associated with adrenal and ovarian failure, suggesting that build up of non-cholesterol sterols can lead to disruption of steroid hormone pathway synthesis, although it should be emphasized that these features are not classically present in most cases of sitosterolemia[36].

Plant Sterol Levels and Normal Physiology

The normal function of ABCG5 and ABCG8 genes is to limit intestinal absorption and promote the biliary excretion of neutral sterols, and in particular limit non-cholesterol sterols (xenosterols). Small, but detectable levels of plant sterols can be found in all normal individuals and these levels are very strongly influenced by genetic inheritance[37, 38]. Common variations in ABCG5 or ABCG8 may contribute to wide inter-individual variation in plasma concentrations of plant sterols among subjects consuming similar amounts of dietary sitosterol. Typically, a 5-fold variation in plasma concentrations of cholesterol precursor sterols and plant sterols can be observed among individuals with normal lipid levels[37, 38]. The effect of genetic and environmental factors on variations in plasma concentrations and sterol-cholesterol ratios of five noncholesterol sterols (cholestanol, desmosterol, lathosterol, campesterol, and sitosterol) was examined[38]. Regression analysis indicated that plasma levels of all five noncholesterol sterols were highly heritable, with the greatest heritable index for sitosterol. Twin studies have confirmed this heritability[37].

Despite this strong heritability, not all of the variations at the *STSL* locus explain the variability in plasma plant sterol levels, suggesting many other loci are likely involved that can affect the low steady-state levels of plasma plant sterols[39].

On the other hand this locus has been implicated in playing a role in susceptibility to gallstone formation in mice and men[40-43], in lipoprotein kinetics in obese individuals[44], in an association with metabolic syndrome marker prevalence[45], in determining plasma LDL concentrations[46], in responsiveness to atorvastatin[47], in responsiveness to plant sterol-fortified margarines, in plasma plant sterol levels as well as cholesterol synthesis rates[48], and in responsiveness to certain diets and plasma lipid levels[49]. The major drawback with the majority of these studies is the small sample size which restricts the power to test the hypothesis that the *STSL* locus plays a role in the measured phenotype (despite the reported significant ‘P’ values). Another potential problem is that there seems to be no purported mechanistic connection made between the putative function of the sterolins and the phenotype that is studied; for example what is the connection between lutein levels and the function of the sterolins[49], or why is the relationship of *STSL* variation seen only in obese persons (which itself is an ‘acquired’ phenotype)[44, 50]? Better designed and well-powered studies that also take into account the haplotype structure of this locus, as well as test mechanistically plausible phenotypes are needed to explore the role of the *STSL* locus in some of these pathways. To date, the strongest data may be for gallstone susceptibility, even though these are still not the level to provide definitive evidence.

Are Plant Sterols a Marker for Premature Atherosclerosis?

Glueck and colleagues were the first to report an association between serum phytosterol levels with a family history of CHD in 595 hypercholesterolemic subjects[51]. In a case-control study conducted in postmenopausal women, those with CAD had elevated ratios of squalene ($p<0.001$), desmosterol ($p=0.005$), campesterol ($p=0.028$) and sitosterol ($p=0.022$) to cholesterol, but had lower respective lathosterol values ($p=0.041$) compared with the

controls[52]. After adjustment for risk factors, the ratios of squalene, lathosterol, campesterol, and sitosterol were significantly ($p<0.05$) associated with an increased risk of CAD. A positive family history of CHD was present among 27 patients but absent among 26 patients undergoing elective coronary artery bypass grafting (Sudhop et al, 2002). Patients with a positive family history for CHD had significantly ($p<0.05$) higher plasma levels of campesterol and sitosterol. Further analysis showed no influence of sex, age, triglycerides, total-, LDL-cholesterol, and HDL-cholesterol, but confirmed a strong influence of plant sterols[53].

The PROCAM Study evaluated the risks of cardiovascular disease associated with sitosterol[54]. In this case-control cohort study, 159 men who had experienced an acute myocardial infarction or sudden death during a 10-year follow-up were matched with controls. Plasma sitosterol concentrations were elevated in cases compared with controls ($p=0.028$). Elevated levels of sitosterol were associated with a 1.8-fold increase in risk ($p<0.05$). Among men at high risk, high sitosterol concentrations were associated with an incremental 3-fold increase in the incidence of coronary events ($p=0.032$), and a significant ($p=0.03$) association between high sitosterol/cholesterol ratio and coronary risk. Plasma sitosterol concentrations were independently predictive of cardiovascular disease. The hazard ratio for sitosterol (1.77) was comparable to that for HDL-cholesterol (1.82) and less than that for LDL-cholesterol (2.90). It was concluded that elevated sitosterol concentrations were associated with an increased risk for major coronary events in high risk men[54].

Not all such studies confirm an association between non-cholesterol sterols and CAD. Results from the Dallas Heart Study were used to determine whether plasma levels of plant sterols were associated with coronary atherosclerosis, as judged by coronary artery calcium measured by EBCT[55]. In 2542 patients aged 30 to 67 years, plasma levels of cholesterol were significantly ($p<0.05$) higher in patients with elevated coronary calcium levels. But plasma levels of sitosterol or campesterol were not associated with elevated coronary calcium. No relationship was observed between a family history of CHD and plant sterol levels, and a similar lack of association was found in a subset of patients with hypercholesterolemia[55]. The EPIC-Norfolk Population study in a prospective nested case-control study consisting of 373 cases and 758 controls, also failed to show a relation ship between sitosterol and CAD[56].

Can We Lower Plant Sterol Levels?

A multicenter, double-blind, randomized, placebo-controlled study examined the effect of ezetimibe on reducing plant sterol levels in patients with sitosterolemia[57]. Among 37 patients who were randomized to placebo or ezetimibe 10 mg/d for 8 weeks, sitosterol plasma levels decreased significantly ($p<0.001$) with ezetimibe (-21% vs. 4% increase with placebo). Campesterol levels also decreased significantly ($p<0.001$) with ezetimibe (-24% with ezetimibe vs. 3% increase with placebo). Total sterol and apolipoprotein B levels also were reduced. In non-sitosterolemic subjects, who have very low levels of plant sterols (<1mg/dL), ezetimibe also led to significant lowering of plant sterols, suggesting that if the entry of plant sterols is blocked via NPC1L1, this can lead to a further lowering in normal as well as sitosterolemia subjects[58].

Summary

Increased knowledge of the defective genes in sitosterolemia expands our understanding of this disease and has shed light into how we regulate dietary sterol absorption, sterol excretion, and keep xenosterols from accumulating in our bodies. This disease has led to the identification of the molecular players in a key physiological step and led to the identification of a previously unknown biochemical pathway that is essential to understanding whole-body sterol balance. Expanding knowledge of the effects of plant sterols and their interplay with cholesterol should

aid the identification of better dietary and pharmacological approaches to manage elevated lipid and lipoprotein levels. In addition, it may also focus attention on how diet, and particular xenosterols, may affect the risk of cardiovascular morbidity and mortality.

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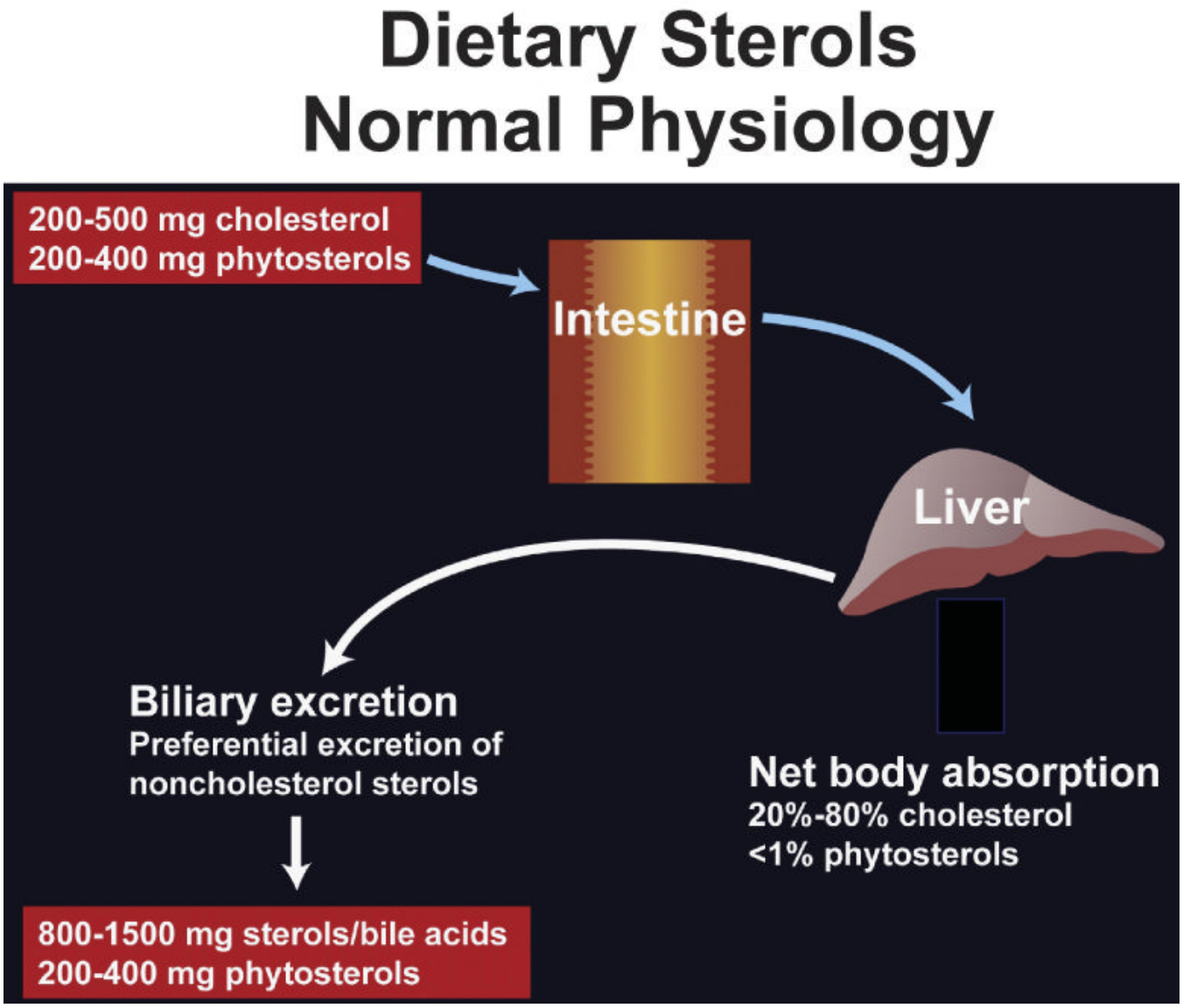

Figure 1. Normal physiology of dietary sterols

Although we ingest an equal amount of plant sterols and cholesterol, the net retention of the plant sterol is <5% on a daily basis, whereas an average of 55% of cholesterol may be absorbed. In addition, the liver excretes a significant amount of sterols, resulting in a net output of more than 1g per day loss in the feces.

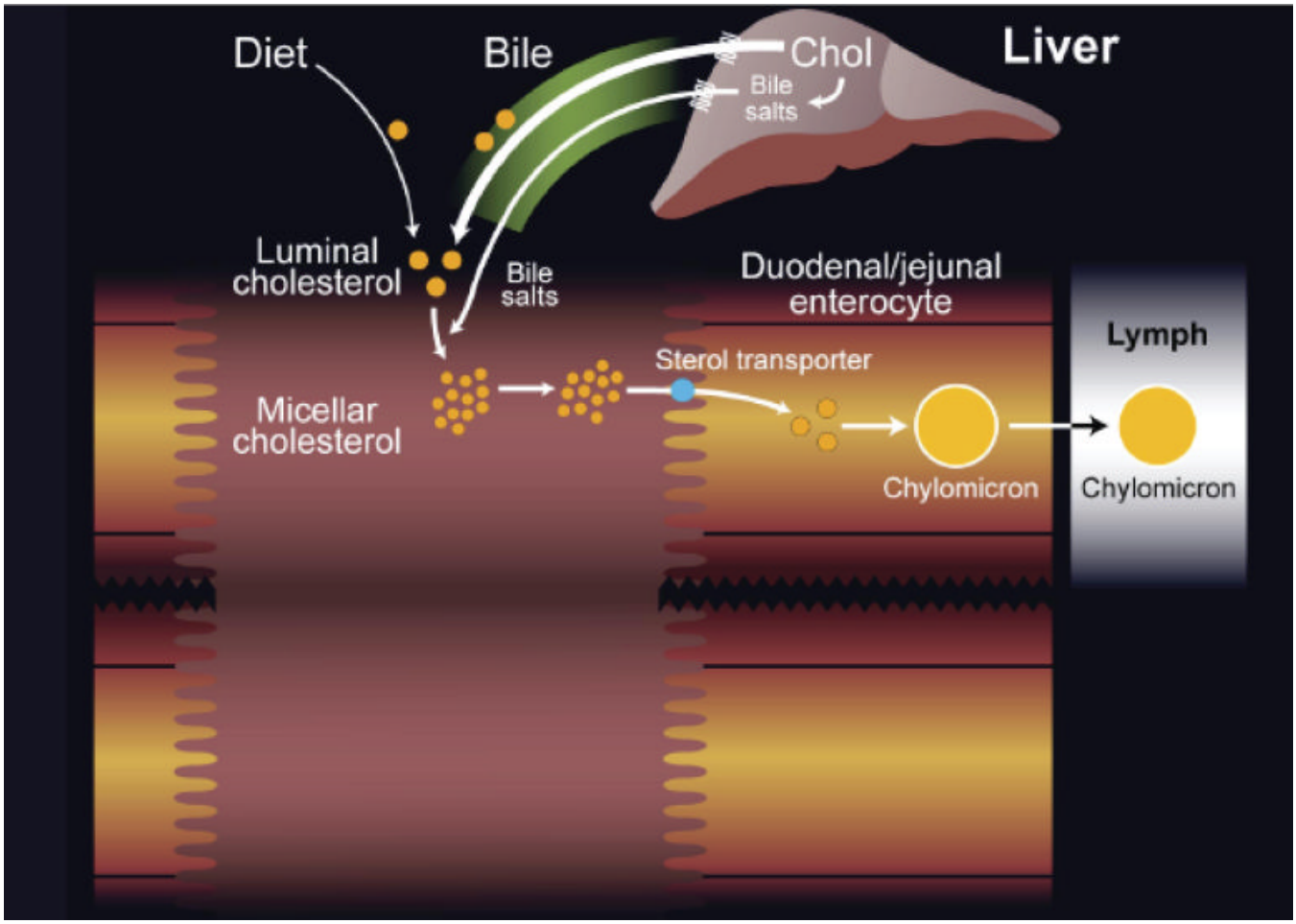

Figure 2. Pathway for intestinal cholesterol absorption

The process of cholesterol (and sterol) absorption begins with the formation of micelles that comprise bile salts and phospholipids present in bile, that allow for neutral sterols to partition into them. Once these micelles are formed, they interact with the apical surface of the enterocyte, leading to the entry of the cholesterol to join the metabolic pool and eventually get secreted as a component of chylomicron particles at the basolateral surface. Note that the bile salts do not enter in the proximal small intestine, but travel further down to the terminal ilieum for specific re-uptake.

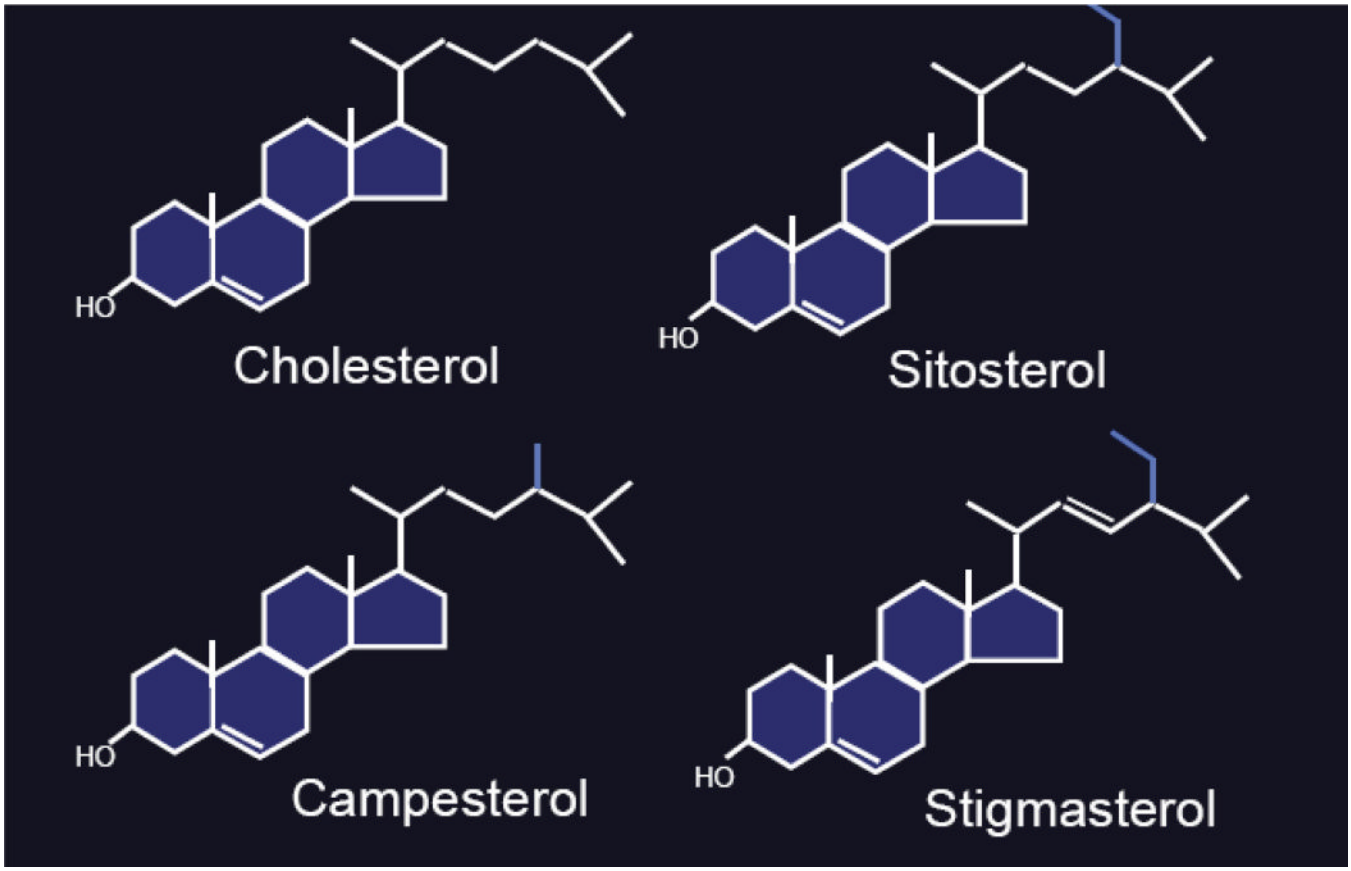

Figure 3. Structures of sterol molecules

The various sterol structures are depicted and show that plant sterols differ primarily in the R tail of the sterol structure and these differences can range from only an extra methyl group (campesterol) to a more complex difference (stigmasterol).

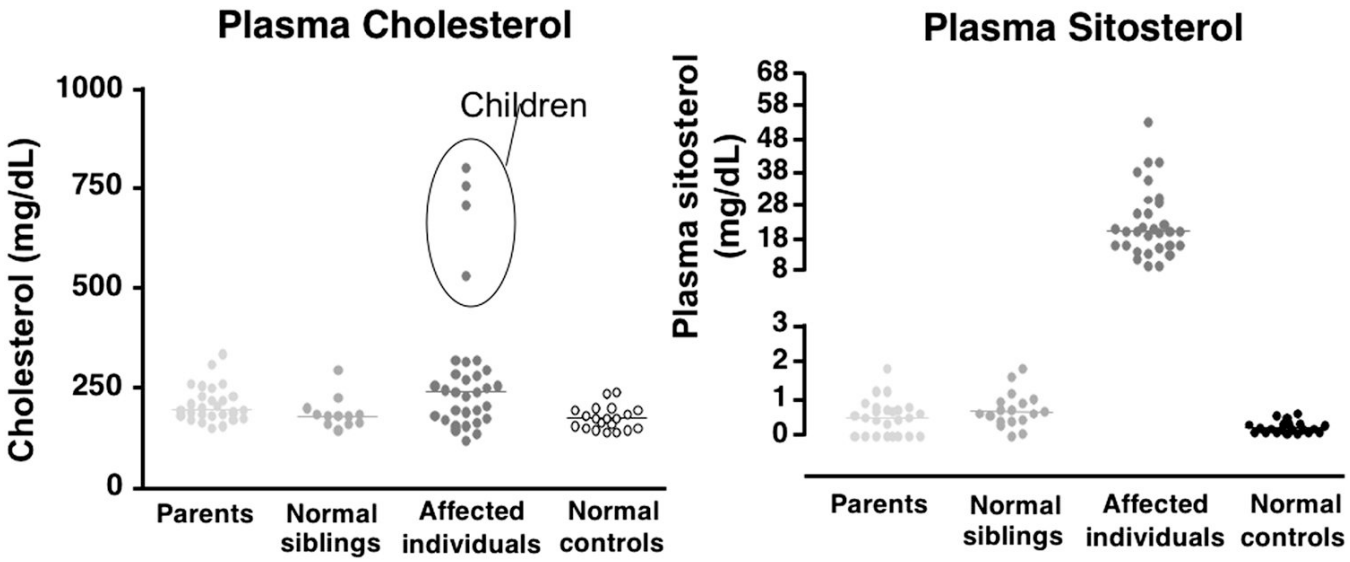

Figure 4. Plasma sitosterol and cholesterol in affected individuals with sitosterolemia and parents, normal siblings, and normal controls

All sterols were determined by either GC or HPLC and show that cholesterol levels were not significantly different in patients with sitosterolemia, except for the 4 subjects (designated by circles in the right hand chart) all of whom had been clinically diagnosed as having ‘pseudohomozygous FH’ but all had sitosterolemia (right hand chart).

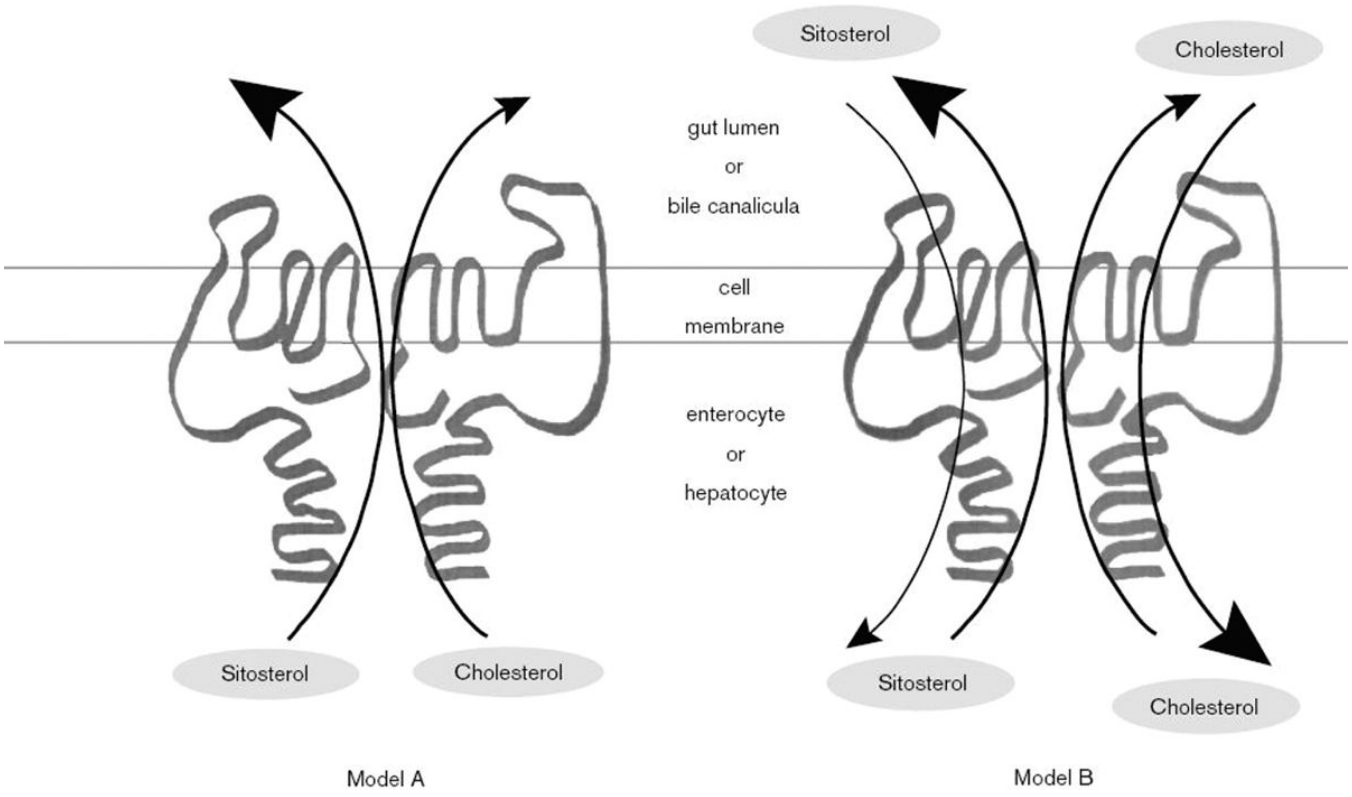

Figure 5. Proposed models for sitosterol and cholesterol metabolism

Based upon the genetics, sterolin 1 and -2 are proposed to function as heterodimers. They could act as extruders, pumping non-cholesterol sterols and cholesterol out (Model A), with a greater affinity to pump non-cholesterol sterol. Alternatively, they could act as channels, allowing sterols in and out of the cell (Model B), but favoring cholesterol in, and plant sterols out. Model A seems to be supported by current experimental evidence.