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# Genetic defenses against noncholesterol sterols

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## Abstract

**Purpose of review—**This review discusses recent progress in the role of ATP-binding cassette proteins ABCG5 and G8 in dietary sterol absorption, excretion and pathogenesis of cardiovascular disease.

**Recent findings—**IFentification of the genetic defect(s) underlying sitosterolemia has led to a renewed interest in the mechanisms of sterol absorption and biliary excretion. Mutations in *ABCG5* (encoding sterolin-1) or *ABCG8* (encoding sterolin-2) cause this disease. These proteins are thought to function by preventing dietary noncholesterol sterols from being retained by the body and for cholesterol excretion into bile.

**Summary—**Despite improvements in treatments for hypercholesterolemia with cholesterol lowering agents, cardiovascular disease still remains highly prevalent. This has prompted many to consider that molecules other than cholesterol may be better biomarkers for this disease and targeting these more directly may allow us to develop more effective therapies. Ideally, if such a biomarker were also the bioactive molecule that is key to initiating/propagating the atherosclerosis pathogenic pathway, this would allow us to develop an optimal predictor and monitor of the disease process. One source of such molecules could come from our diet, with potential candidates such as noncholesterol sterols, oxysterols, oxidized sterols or some as yet unidentified dietary bioactive molecule. Nature has evolved a protective mechanism by which such molecules are kept out of the body, thereby reducing the negative effects of these compounds. The newly identified sterolin proteins involved in the absorption and excretion of dietary sterols may fit this bill. If so, we would speculate that a better biomarker may be lurking within their substrate specificities.

### Keywords

sitosterol; plant sterols; ABCG5; ABCG8; intestine

### Abbreviations

**ABCG5** ATP-binding cassette G5; **ABCG8** ATP-binding cassette G8; **CHD** coronary heart disease; ***STSL*** sitosterolemia locus

## Introduction

Cardiovascular disease remains the leading cause of morbidity and death in industrialized societies, despite advances in therapies to treat this complicated disease process. The use of inhibitors of de-novo cholesterol synthesis, namely hydroxymethyl glutaryl coenzyme A reductase inhibitors or statins, has had a significant impact on reducing the risk of

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cardiovascular events as shown by multiple trials (4S, CARE, LIPID, HPS etc.) [1•]. Despite the increasing use of statin therapy to treat abnormal lipids, especially among patients with diabetes and patients with previous history of cardiovascular disease, the prevalence of cardiovascular disease has not dramatically decreased and still remains high. Statin therapy inhibits the synthesis of de-novo cholesterol, but has no effect on the preexisting cholesterol that arises from dietary or other sources. Additionally, while cholesterol is associated with cardiovascular disease, it is only a biomarker, not the bioactive molecule of atherosclerosis. By this we mean that cholesterol per se is not responsible for setting into motion the biochemical changes that lead to the pathogenesis of atherosclerosis. Many bioactive molecules, ranging from oxysterols to oxidized sterol and lipids, have been proposed as such candidates, but the 'smoking gun' of atherosclerosis has yet to be definitively identified. These observations have prompted many investigators to look at other areas of cholesterol metabolism, bile acid metabolism and dietary substances that may either provide a better biomarker than cholesterol, or even the bioactive molecule that is key to initiating or maintaining the atherosclerotic process.

This review will focus on the recent progress in the area of dietary sterol absorption and the possibility that dietary noncholesterol sterols or their metabolites may be causing atherosclerosis.

## Background on dietary sterols

A western diet contains around 400 mg of cholesterol per day, derived from animal sources, and about 200–400 mg of noncholesterol sterols, derived mostly from plants [2,3]. On average, about 55% of the dietary cholesterol is absorbed and retained on a daily basis, but almost none of the noncholesterol sterols (such as plant sterols sitosterol, campesterol, brassicasterol etc.) are retained [4]. While most of the total body pool of cholesterol is derived from de-novo cholesterol synthesis, it is now clear that dietary sources play a crucial role in maintaining total body sterol balance. Influx of cholesterol from the diet regulates the amount of de-novo synthesis. The liver is a key organ in maintaining this balance. Excess body cholesterol, derived from endogenous synthesis or from dietary absorption, is excreted exclusively by the liver, either by direct excretion as free cholesterol into bile, or by breakdown to bile acids and excreted as bile acid conjugates into bile. A small amount of dietary non-cholesterols do enter the body, but these are rapidly excreted by the liver into bile, almost unchanged, thus resulting in a net very low daily absorption [5]. Why does the body selectively exclude these noncholesterol sterols? One possible hypothesis has been that some molecule/metabolite from the noncholesterol sterol category is somehow involved directly, acting as a bioactive molecule for the pathogenesis of atherosclerosis. Molecules that may fit this bill are the plant sterols, oxidized cholesterol, as well as other metabolites. For the purposes of argument, we will restrict our discussion to plant sterols, without suggesting that the other molecules are not important. Plant sterols are normally present in very low amounts in the blood and thus are at least 100-fold less abundant than cholesterol [6,7]. This makes them better bioactive molecules than cholesterol, though their concentrations are still at least another 10–100-fold higher than one would expect for a bioactive molecule (i.e. concentrations of hormones etc.). There is evidence that the levels of plant sterols may predict cardiovascular disease, and response to statins [8, 9•,10].

Now with the identification of the molecular defect(s) that cause a rare disorder affecting this pathway, this area of investigation has received renewed vigor and interest. Based upon the disrupted physiology, the proteins involved are now hypothesized to regulate the selective absorption of cholesterol and in excluding noncholesterol sterols in the intestine.

## A model for studying sterol absorption

It is well known that dietary sterols are absorbed by the intestine and secreted into the bile, but the exact mechanisms for this process were unclear. However, the identification of a rare autosomal recessive disorder of sterol metabolism termed sitosterolemia also known as phytosterolemia (MIM 210250) has helped to shed light on the processes by which sterols are absorbed and processed. The clinical presentation includes tendon xanthomas (usually involving the Achilles tendon), hemolytic episodes, arthritis/arthralgias, and most strikingly, accelerated atherosclerosis [11,12]. Diagnosis is confirmed by showing increased plasma and tissue levels of plant sterols [11–13]. Sitosterolemia was mapped to the sitosterolemia (*STSL*) locus on human chromosome 2p21 [14,15]. The *STSL* locus comprises two genes, *ABCG5* and *ABCG8*, encoding sterolin-1 and sterolin-2, respectively, and mutations in either one of these genes causes sitosterolemia [16–18]. In the absence of functioning sterolin-1 (ABCG5) or sterolin-2 (ABCG8), patients hyper-absorb all non-cholesterols, retain these in the body and show a failure to excrete these sterols into bile [5,19–22]. Interestingly, in these patients cholesterol is also hyper-absorbed from the diet and the bile is also significantly cholesterol depleted. Additionally, in the liver, the sterolins are also responsible for the rapid excretion of noncholesterol sterols into bile and thus ridding the body of these molecules. Current knowledge indicates that the sterolins may also be one of the putative cholesterol pumps long sought after which operate at the apical surface of hepatocytes. Based upon all of these data, one lead investigator in this field has suggested that the *STSL* locus is a genetic defense against cholesterol (H. Hobbs, personal communication), and we propose that this locus may also be a defense against a bioactive noncholesterol sterol.

Since the discovery of the genetic basis for this disease and the identification of two ATP-binding cassette half transporters involved in sitosterolemia (ABCG5/ABCG8) there has been a renewed interest in the function, regulation, and inter-play among plant sterols and cholesterol. A mouse model, disrupting both the genes that encode for ABCG5 and ABCG8 simultaneously, results in a phenotype similar to the human disease [23••]. It is important to note that a mutation in both alleles of either ABCG5 or ABCG8, but not both genes, in the human results in disease. While the double knockout mouse model is valuable in showing the need for the two transporters, it remains to be seen whether or not a single knockout of ABCG5 or ABCG8 in mice will show the same phenotype as the human disease. Interestingly, overexpression of human ABCG5/ABCG8 in a transgenic mouse model results in an increased biliary cholesterol secretion, a 50% reduction in the fractional absorption of dietary cholesterol and a reduction of plant sterols [24•]. These studies support the view that ABCG5/ABCG8 somehow work as pumps in the intestine and liver to selectively move cholesterol and noncholesterol sterols from the enterocyte/bile cannaliculus back to the lumen. The exact mechanism by which these transporters work, whether they are flipases or ATP-dependent pumps, has yet to be determined [25,26]. Additionally, it is not clear if sterolin-1 and sterolin-2 form obligate heterodimers, as is suggested by the genetics as well as by in-vitro data [27•], or whether they can have independent function as well. When mice were fed diets high in cholesterol, mRNA expression of ABCG5/ABCG8 increased approximately threefold in the liver and approximately twofold in the intestine compared with those mice fed a regular chow diet [28••]. Thus, these genes respond to environmental dietary sterols, although whether they are also increased by high phytosterols has yet to determined.

As has been previously shown, plant sterols are capable of reducing total cholesterol levels [29–32]. Based upon the current literature it is now possible to develop a model of how this is accomplished (Fig. 1). Plant sterols and cholesterol from the diet need to be solubilized in micelles for absorption. Once these are formed in the intestinal lumen the sterol-laden micelle interacts with the intestinal brush border and sterols enter the enterocyte by a yet unknown transport process. Within the enterocyte cholesterol is selectively esterified by *ACAT2* and

packaged into chylomicrons at the basolateral membrane to be transported via lymphatics. The remaining free cholesterol and plant sterols are presumed to be 'pumped out' of the enterocyte back to the intestinal lumen by the complex of ABCG5/ABCG8. Plant sterols are effective in reducing dietary cholesterol absorption because they displace cholesterol from the micelle thus making less free cholesterol available for absorption into the cell. Any plant sterols that enter the enterocyte are largely excluded, presumably by these sterolins, from being packaged into chylomicrons. What little plant sterols that do make it through into chylomicrons enter the lymph, only to be rapidly cleared by the liver into bile. In this context, based upon genetic analyses of mice, Sehayek *et al*. [33••] have implicated yet another genetic locus, distinct from the *STSL* locus, that may also determine the level of plant sterols. Though this is only a putative locus, the identity of this locus is also likely to generate some considerable interest. In the liver the ABCG5/ABCG8 complex is presumed to operate in a fashion similar to that proposed for the intestine. Localized to the apical surface of the hepatocytes, they are thought to 'pump' excess free cholesterol as well as the plant sterols into the bile to be excreted.

## Role of phytosterols in cardiovascular disease

The concept that elevated plant sterols are a risk factor for coronary heart disease (CHD) was first suggested by Glueck *et al*. [10]. In a study of 595 patients they found that hyperphytosterolemia was a heritable marker among families for hypercholesterolemia and thus increased risk for CHD. Do variations at the *STSL* locus play a role in regulating plant sterol levels or predisposition to CHD? In an examination of nuclear families, Berge and his colleagues [34••] addressed this issue. They presented evidence that the plasma plant sterol levels were influenced by heritability, and further implicated specific variations at the *STSL* locus. In this context, patients with known CHD and a positive family history of CHD had plant sterol levels that were 30% higher compared with patients with CHD, but no significant family history of CHD [9•]. When taken in context with the increased premature CHD in many of the patients with sitosterolemia, who have mutations at the *STSL* locus, this strengthens the case for considering the concept of a dietary noncholesterol sterol as a source of chronic environmental toxin. To be bold and take it one step further it can be argued that it is not plant sterols that cause disease but some other noncholesterol sterol like molecule in the diet that is absorbed along with these sterols that is the true bioactive molecule causing atherosclerosis. It could then be said that plant sterols are again only biomarkers of disease just as cholesterol is only likely to be a biomarker of disease. It remains to be seen if any of these concepts will come to fruition. While plasma plant sterol levels may not be the ideal biomarkers to evaluate CHD risks (assays are both complex and costly and there are insufficient data to support their usefulness), a biomarker that is significantly better than cholesterol needs to be developed. To date, we have made many incremental steps towards this goal (C-reactive protein, homocysteine, IL-6 etc.), but the single, simple highly informative test eludes us. Until then, cholesterol will remain the best biomarker we have.

## Conclusion

Many questions still remain as to how cholesterol and other sterols play such a significant role in cardiovascular disease. Noncholesterol sterols (primarily, but not exclusively, plant sterol in our diets) or their metabolites may be the bioactive compounds that mediate atherosclerosis. They are good candidates since they are in such low – almost immeasurable – concentrations in normal controls, and we have evolved specific molecular mechanisms to exclude these from our bodies. Examination of at-risk populations for variations in these newer biomarkers and the investigation of the genetics of these are likely to yield useful information. The elucidation of a rare genetic defect has opened an even larger field of the role of the *STSL* locus and its proteins, the sterolins, on the risk of cardiovascular disease. Hopefully, the old adage, that the way to a man or woman's heart is through his or her stomach, is really not true.

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reveals variations in plant sterol levels is highly heritable due to polymorphisms in the ABCG8 and likely ABCG5.

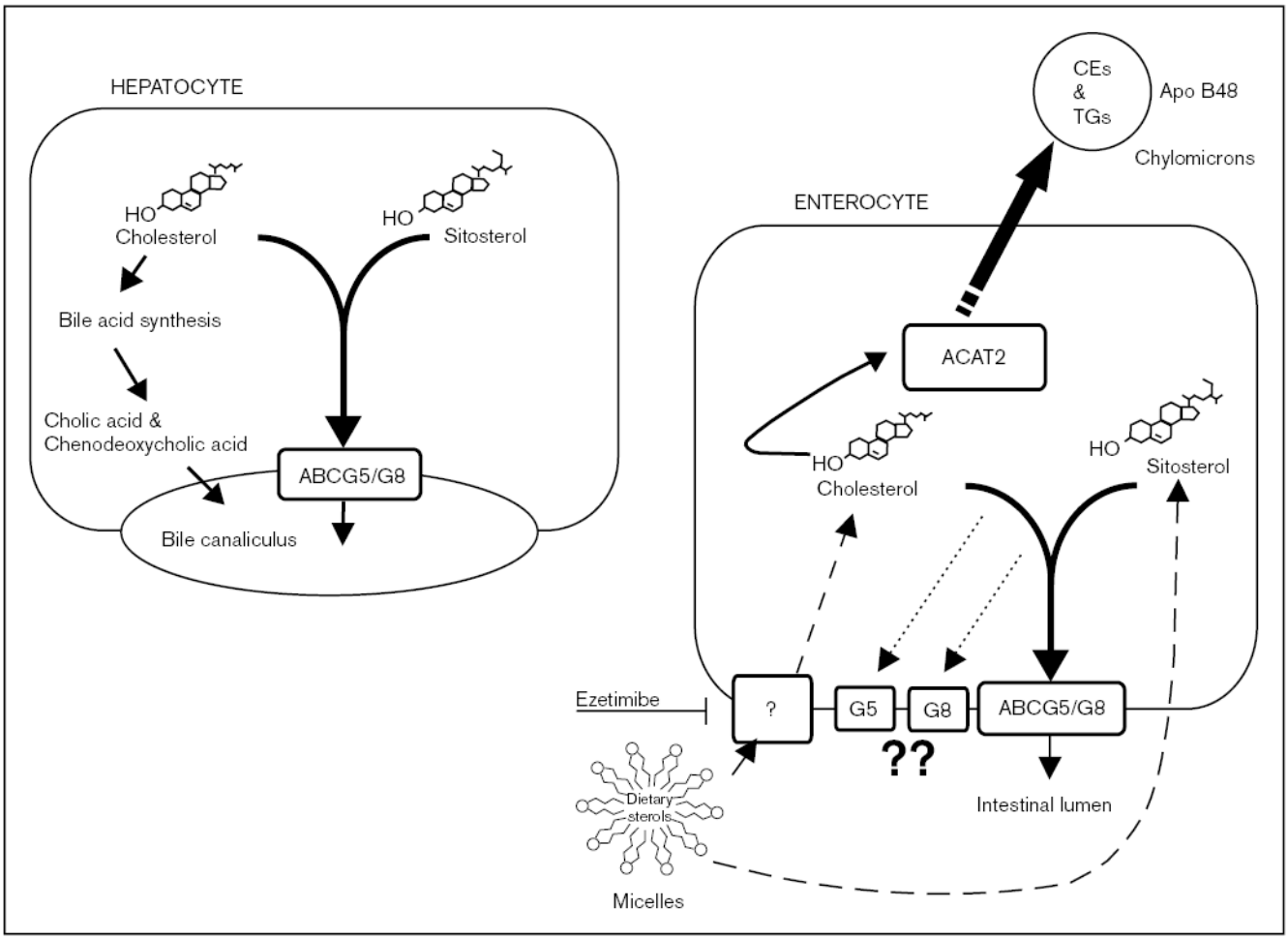


**Figure 1. Possible mechanism for ATP-binding cassette G5/G8 (ABCG5/G8) function in the hepatocyte and enterocyte**

In the hepatocyte, ABCG5/G8 ‘pumps out’ free cholesterol and noncholesterol sterols into the bile. In the enterocyte ABCG5/G8 may function as hetero or homodimers as shown to ‘pump out’ free cholesterol and noncholesterol sterols back into the intestinal lumen. The recently released cholesterol-lowering drug Ezetimibe somehow blocks the direct absorption of micellar cholesterol. ACAT, acyl-coenzyme A: cholesterol acyltransferase; Apo, apolipoprotein; CE, cholesteryl ester; TG, triglyceride.