

Phytosterols and vascular disease

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Purpose of review

Phytosterols and stanols are plant derivatives that compete with cholesterol for intestinal absorption and thereby lower serum cholesterol concentrations. They have been developed as food additives to help lower serum cholesterol but there is concern that these additives could inadvertently increase cardiovascular risk. This concern arises from the observation that patients with the rare genetic condition phytosterolemia overabsorb phytosterols and develop premature atherosclerosis. This review evaluates the relationship between phytosterol and stanol supplementation and cardiovascular risk.

Recent findings

Plant sterol supplementation produces minimal increases in blood phytosterol concentrations in humans. Recent animal studies suggest that phytosterols reduce atherosclerosis in the Apo-E deficient mouse model. The evidence from human studies is mixed and does not prove or disprove an increase in atherosclerotic risk from serum phytosterol levels. An increase in risk seems unlikely, but additional studies should address this possibility.

Summary

Phytosterols are effective in lowering low-density lipoprotein-cholesterol levels, and do not appear to increase atherosclerotic risk, but additional research on this topic is necessary.

Keywords

atherosclerosis, atherosclerotic cardiovascular disease, cholesterol, coronary artery disease phytosterolemia, phytosterols

Introduction

The remarkable reductions in low-density lipoprotein cholesterol (LDL-C) levels produced by the hydroxyl-methyl-glutaryl coenzyme-A (HMG CoA) reductase inhibitors or statins have revolutionized the treatment of atherosclerotic cardiovascular disease (ASCVD) [1]. Nevertheless, these agents reduce the incidence of ASCVD by only approximately 30% [2], warranting a search for complementary approaches both to reduce LDL-C concentrations and ASCVD risk.

Plant sterols and their saturated derivatives, stanols, reduce LDL-C by competitively inhibiting intestinal cholesterol absorption and can be used alone or with statins and other medications ostensibly to reduce ASCVD risk. Despite such putatively beneficial effects in lowering LDL-C concentrations, there are three areas of concern regarding their widespread use. First, rare individuals with the disease phytosterolemia overabsorb plant sterols and develop premature ASCVD [3]. Second, phytosterols have been detected in atherosclerotic lesions from individuals with apparently normal cholesterol absorption [4]. Third, some studies suggest that elevated serum concentrations of plant sterols are associated with increased ASCVD disease [5,6]. This review examines the evidence linking phytosterols supplementation and ASCVD.

Methods

The medical literature was systematically searched through to 31 August 2006 using PubMed and Medline as the search sources and the word combinations phytosterols and atherosclerosis, sitosterol and atherosclerosis, phytosterols. Relevant articles were reviewed by the primary author and included if pertinent to the topic.

Phytosterols

The phytosterols (including sitosterol, campesterol, and bressicasterol) and their saturated derivatives, the stanols, are naturally occurring plant derivatives. Stanols are less abundant in nature. The primary sources of phytosterols are vegetables, nuts, fruits and seeds. Seeds contain an average of 120 mg of plant sterols/100 g wet weight, vegetables contain 20 mg/100 g of wet weight and fruit about 15 mg/100 g wet weight. Sitosterol, campesterol and stigmasterol are most abundant in nature comprising 65%, 30%, and 3% of dietary phytosterol intake [7].

Phytosterols and cholesterol are structurally similar but are metabolized differently. The average western

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Abbreviations

ABC ATP binding cassette
CAD coronary artery disease
GLC gas-liquid chromatography
LDL-C low-density lipoprotein cholesterol

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diet approximately contains 200–500 mg of cholesterol, approximately 200–400 mg of plant sterols and 20–50 mg of plant stanols [8]. Humans absorb 55–60% of dietary cholesterol and less than 5% of phytosterols [9–11].

Absorption of cholesterol and phytosterols

In the intestine, plant sterols are initially solubilized into a micelle form. These micelles interact with brush border cells and are transferred into enterocytes. Plant sterols are esterified within the enterocyte, assembled into chylomicrons and secreted into the lymphatics. They are excreted via the biliary system. The nonesterified phytosterols are transported back into the intestinal lumen by sterolin (1 and 2) pumps containing the ATP binding cassette (ABC) proteins encoded by the genes *ABCG5* and *ABCG8*. These are expressed in the mucosal cells and the canalicular membrane, and they resecret sterols, especially absorbed plant sterols, back into the intestinal lumen and from the liver into bile [12]. Defects of either of these co-transporters lead to the rare inherited disease of phytosterolemia. The molecular mechanisms responsible for the transfer into the enterocyte are not fully elucidated. Recently it has been revealed that the Niemann–Pick C1-like 1 (NPC1L1) transporter is most likely responsible for the transport of cholesterol and plant sterols from the brush border membrane into the intestinal mucosa. Ezetimibe interferes with NPC1L1, reducing the intestinal uptake of cholesterol and plant sterols [13,14]. Stanols are absorbed less than sterols (0–3%) and increasing the length of the side chain of phytosterols increases hydrophobicity and decreases absorption [15]. Because of their lower intestinal absorption and preferential biliary excretion, plant sterols comprise less than 1% of total circulating sterols in humans [16]. Unabsorbed sterols undergo transformation by intestinal microflora to produce metabolites such as coprosterol and coprostanone [17,18]. The standard colorimetric and enzymatic methods used to measure blood cholesterol identify the double bonds between C5 and C6 and 3 β -hydroxy bonds respectively. Since phytosterols also contain these bonds conventional cholesterol measurements do not distinguish between cholesterol and phytosterols. To be correctly identified, phytosterol and stanol concentrations must be measured by gas liquid chromatography (GLC) or high performance liquid chromatography (HPLC) [19].

Phytosterolemia

Phytosterolemia is a rare autosomal recessive disorder, characterized by markedly increased tissue and plasma sterol concentrations leading to premature ASCVD. Phytosterolemia was first described by Bhattarcharya and Connor in 1974 in two sisters who presented with xanthomatosis, normal cholesterol levels, and, elevated sitosterol levels. Their sitosterol levels were 27.1 mg/dl and 17.7 mg/dl, comprising 11% and 16% of their circulating

sterols versus the usual level of below 1% [20]. Phytosterolemia patients present at a young age with tendon xanthomas similar to familial hypercholesterolemia and clinical symptoms produced by premature atherosclerosis including angina, myocardial infarction and sudden death [3]. Non-cardiac abnormalities include abnormal red cells, hemolysis, thrombocytopenia and abnormal liver function tests [21]. The primary difference between familial hypercholesterolemia and phytosterolemia is that the latter has normal or slightly elevated cholesterol levels, but a high ratio of serum plant sterol to cholesterol. In one of the above-mentioned patients the tendon xanthomas had a plant sterol content of only 17.5% [20]. This raises the possibility that phytosterols facilitate the deposition of other material in extravascular locations.

Genetics of phytosterolemia

Phytosterolemia is an autosomal recessive disorder with an incidence of one in five million people. Homozygosity (but not heterozygosity) for defects in either *ABCG5* or *ABCG8* genes is implicated. These genetic defects eliminate the reverse transport of phytosterols into intestinal lumen, increase phytosterol absorption, decrease biliary excretion and ultimately lead to phytosterolemia.

The *ABCG5* and *ABCG8* genes are located on chromosome 2p21 [22]. These genes have been mapped in Amish-Mennonite, Finnish, Indian and Japanese families [23]. Heterozygotes for functional defects in *ABCG5* or *ABCG8* do not manifest clinical symptoms. They do have increased phytosterol absorption, but normal serum levels because of rapid biliary excretion [24,25].

Phytosterolemia diagnosis and management

The diagnosis of phytosterolemia should be considered in young patients presenting with tendon xanthomas, and evidence of premature atherosclerosis [3]. Their total cholesterol concentrations may be slightly elevated, and they may have no family history of hypercholesterolemia. Since routine enzymatic and colorimetric assays measure phytosterols as cholesterol, GLC or HPLC is required to distinguish phytosterols from cholesterol [19]. Historically the treatment of phytosterolemia included a diet restricted in cholesterol and plant sterols, bile salt binding resins, ileal bypass surgery and plasmapheresis [26,27], but ezetimibe has revolutionized treatment because it directly impedes sterol absorption [28,29]. Statins tend to increase phytosterol concentrations possibly by increasing sterol absorption [30]. In phytosterolemia the activity of HMG CoA reductase is low and membrane expression of the hepatocyte LDL receptor is increased, so statins are less effective in reducing cholesterol levels [31].

The clinical use of phytosterols

Plant sterols are used to lower the serum cholesterol levels. They decrease the cholesterol absorption of plant sterols and were documented to lower serum cholesterol as early as 1951 by Peterson, who fed chicks with plant sterols [32]. In 1957, Eli Lilly introduced sitosterol as a cholesterol lowering agent called Cytellin. Because of its poor water solubility and poor bioavailability, it was not highly effective or profitable and was taken off the market. Esterification of plant stanols with fatty acids convert them from a crystalline powder with low lipid solubility into fatty substances that can be incorporated into a variety of foods [33]. This property allows them to be used as additives in fatty foods such as Benecol (Raisio, Finland; McNeil Nutritionals LLC, Ft. Washington Pennsylvania, USA) and Take Control (Unilever, London, UK) margarines, which contain plant stanol and sterol esters, respectively. The reduction of LDL-C obtained by phytosterols and stanols in doses of 0.7 g/day to 2.5 g/day ranges from 6.7% to 11.3% [34]. This effect is additive to the effect of statins and the addition of 5.1 g/day of phytosterol to statins produces an additional 15% reduction in LDL-C concentration [35]. Both phytosterols and stanols are well tolerated and have few side effects [36].

Phytosterols, atherosclerosis and vascular disease

The presence of premature ASCVD in individuals with phytosterolemia raises the possibility that even mild increases in serum phytosterol concentrations may be atherogenic. This has important implications because phytosterols are increasingly used as a dietary component to lower serum cholesterol and they produce small increase in serum phytosterol levels, about 0.6–2 mg/dl [37]. The possibility that phytosterols increase ASCVD risk has been examined using animal models, tissue measurements, and cross-sectional comparisons of patients with and without ASCVD.

Animal models

Moghadasian *et al.* [38] compared a standard western chow diet with or without phytosterols derived from tall oil (phytosterol mixture) in Apo-E deficient mice. They found reduced atherosclerotic lesions in the phytosterol fed mice. In the same Apo-E deficient mouse model, these investigators compared control fed animals with

animals provided with either phytosterol or probucol. Compared to the controls, probucol given mice had accelerated atherosclerosis by threefold. Phytosterol-treated animals had half the disease of the controls [39]. These same authors have also used the Apo-E deficient mouse model to document that phytosterols reduce progression of established atherosclerotic lesions. Apo-E deficient mice were fed western diets for 14 weeks to induce atherosclerosis and subsequently fed them a diet enriched with phytosterols for an additional 25 weeks. Atherosclerotic lesion progression was reduced in the plant sterol treated group (28% versus 40%) [40]. Again using similar Apo-E deficient mice these investigators compared the progression of atherosclerosis in controls, with groups treated with phytosterol, phytosterol and cyclosporin, or cyclosporin [41]. Atherosclerotic lesion size was least in the phytosterol-treated animals (0.15 mm², mean), intermediate in the combination therapy group (0.22 mm², mean) and greatest (0.41 and 0.42 mm², means) in the control and cyclosporine-treatment groups. Similar differences were also observed in lesion/artery lumen ratios (Table 1).

Results from Plat *et al.* [42**] also suggest that plant sterols and stanols are not atherogenic. These authors studied the effect of atorvastatin-induced increases in serum plant sterol and stanol concentrations on the size and severity of atherosclerotic lesions in heterozygous LDL receptor deficient mice. Atherosclerotic lesion development was assessed in controls (fed western diets alone) compared with five groups fed diets enriched with either atorvastatin; plant sterols; plant stanols; atorvastatin and plant sterols; or atorvastatin and plant stanols. Atorvastatin induced elevations of plant sterols and stanols did not accelerate atherogenesis (Fig. 1). In a companion study, atherosclerosis was induced in the mice before 12 weeks of treatment with sterol esters, stanols esters or atorvastatin. Compared to controls, animals treated with the sterols and stanols showed regression of the atherosclerotic lesions by 66 and 64%, respectively (Fig. 2).

Human studies

There are at least seven studies that have examined the possibility that phytosterols may contribute to ASCVD risk. Glueck *et al.* [43] were the first to suggest that elevated phytosterols may be a risk factor for coronary

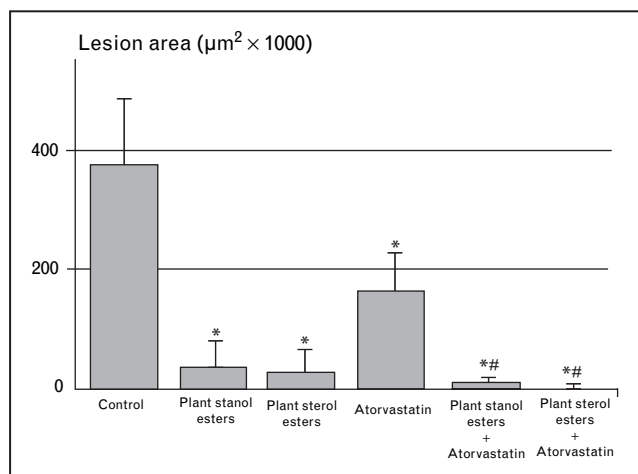
Table 1 Morphometric features of atherosclerotic lesions in apo-E knockout mice treated with cyclosporine or phytosterols or both

Group	<i>n</i>	Lesion size (mm ²)	Lumen area (mm ²)	Lesion/lumen ratio
Control	7	0.42 ± 0.14	1.26 ± 0.22	0.32 ± 0.07
Phytosterol	8	0.15 ± 0.08*	1.19 ± 0.12*	0.13 ± 0.06*
Phytosterol + cyclosporine	8	0.22 ± 0.08*	1.15 ± 0.19*	0.18 ± 0.05*
Cyclosporine-treated	7	0.41 ± 0.09	1.31 ± 0.19	0.31 ± 0.05

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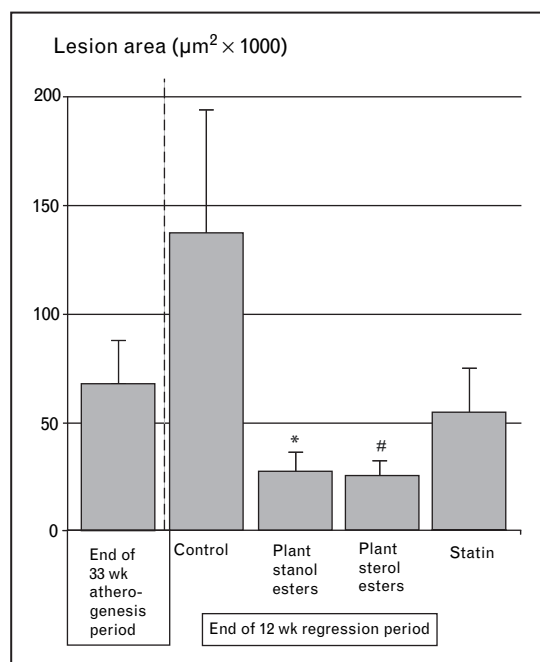
* *P* < 0.05 as compared to either controls or cyclosporine-treated group.

Figure 1 Lesion areas in female heterozygous LDL receptor deficient mice



Mice were fed plants stanols (1% w/w), plant sterols (1% w/w), atorvastatin (0.0025% w/w), plant stanols (1% w/w) plus atorvastatin (0.0025% w/w) or plant sterols (1% w/w) plus atorvastatin (0.0025% w/w) for 35 weeks. Plant sterols and stanols were fed as fatty acid esters. **P* < 0.001 versus control; #*P* < 0.05 versus atorvastatin. Reproduced with permission [42].

Figure 2 Lesion areas in female heterozygous LDL receptor deficient mice



Mice were fed with Western type control diet for 33 weeks (atherogenic period) followed by a 12-weeks period (regression period) in which animals were fed with plant stanols (2% w/w) sterols (2% w/w) or atorvastatin (0.005% w/w). **P* = 0.016 versus controls and #*P* = 0.026 versus controls. Reproduced with permission [42].

artery disease (CAD). These authors measured serum cholesterol as well as phytosterols by GLC and thin layer chromatography in 595 patients with hypercholesterolemia. Elevated phytosterols levels were associated with a family history of CAD suggesting that slightly elevated phytosterols were a heritable risk factor.

Sutherland and Williams [44] in 1998 examined the association of plant sterol levels and angiographically determined CAD in 44 patients with similar cholesterol levels. The severity of CAD was inversely related to the ratio of plasma lathosterol to sitosterol. Since lathosterol is an indicator of cholesterol production and sitosterol an indicator of sterol absorption, the inverse relationship suggests that high plant sterol absorption is related to increased CAD severity. Increased plant sterol absorption, however, may also be an indicator of increased cholesterol absorption; hence increased phytosterol absorption is often secondary to inhibition of cholesterol synthesis that in turn stimulates cholesterol absorption.

Rajaratnam *et al.* [45] measured serum squalene, desmosterol and lathosterol levels (as indicators of cholesterol synthesis), and serum cholestanol, campesterol and sitosterol levels (as indicators of cholesterol absorption) in 48 postmenopausal women with angiographically verified CAD and in 61 controls. The CAD patients had a higher ratio of plant sterols to cholesterol and decreased ratio of lathosterol to cholesterol suggesting that low cholesterol synthesis may cause increased plant sterol absorption and may increase CAD risk, although as stated above this may have also indicated increased absorption of cholesterol. Unfortunately, there was no attempt to assess occult CAD in the controls.

Sudhop *et al.* [6] compared phytosterol levels in 53 patients undergoing coronary artery bypass graft surgery. Phytosterol levels were increased (campesterol 0.50 µmol/l versus 0.38 µmol/l, *P* = 0.001; sitosterol 0.40 µmol/l versus 0.11 µmol/l, *P* = 0.004) among patients with a family history of CAD suggesting that genetically increased phytosterol absorption may increase CAD risk.

Miettinen *et al.* [4] measured total cholesterol, esterified cholesterol, and phytosterol levels in serum and arterial tissue obtained from patients undergoing carotid endarterectomy (*n* = 25). Those patients with a higher ratio of absorption of plant sterols to cholesterol had a corresponding higher ratio of phytosterols to cholesterol in atherosclerotic arterial tissues, suggesting that increased phytosterol absorption does contribute to development of atherosclerotic lesions.

The Prospective Cardiovascular Munster (PROCAM) study is a nested, case-control, 10-year follow-up study of a random sample of the Munster population. Sitosterol

levels in men with a CAD event ($n = 159$) were compared with controls without CAD ($n = 318$). Patients with sitosterol levels of $5.25 \mu\text{mol/l}$ had a 1.8-fold increase in CAD risk when compared to controls whose average sitosterol level was $4.27 \pm 2.38 \mu\text{mol/l}$ ($P < 0.05$). Among high risk subjects, defined as having an estimated global CAD risk of greater than 20% in 10 years, serum sitosterol levels were higher ($P = 0.032$) and CAD risk increased threefold [5].

The Dallas Heart Study measured plasma cholesterol, sitosterol and campesterol levels in 3254 subjects, aged 30–65 years obtained from a probability based sample of Dallas County, Texas. They compared the mean levels of plant sterols in individuals with a plasma level of cholesterol of more than 240 mg/dl, and a positive family history of CAD to those with no family history of CAD. No significant differences in plasma sitosterol ($0.16 \pm 0.003 \text{ mg/dl}$ versus $0.16 \pm 0.004 \text{ mg/dl}$) or campesterol ($0.26 \pm 0.004 \text{ mg/dl}$ versus $0.27 \pm 0.005 \text{ mg/dl}$) were found between the two groups. These results suggest that phytosterol levels do not contribute to familial coronary heart disease [46].

This study also compared plasma cholesterol, sitosterol, and, campesterol levels in 2542 subjects who underwent coronary artery calcification scoring using electron beam computerized tomography. Subjects with a positive scan had higher levels of cholesterol than comparison subjects, but similar levels of sitosterol and campesterol [46].

Conclusion

The fact that increased plant sterol levels in phytosterolemia are associated with atherosclerosis and the presence of phytosterols in atherosclerotic tissue raises the possibility that increased absorption of these compounds can increase ASCVD risk. Furthermore, the observation that phytosterols comprise a relatively small part of xanthomata in phytosterolemic patients raises this possibility that phytosterols aggressively promote lesion formation, and that their risk may outweigh their beneficial effect on lipid levels. There is intense interest in phytosterols as an atherosclerotic risk factor because these agents are used as food additives to reduce LDL-C levels. Esterification reduces their absorbability, but does not eliminate their absorption.

Available animal studies suggest that phytosterols reduce atherosclerosis in the Apo-E deficient mouse model. Human studies are mixed, and do not prove or disprove an increase in atherosclerotic risk that can be clearly related to serum phytosterol levels. It is reassuring that vegetarians who consume considerable plant sterols are at decreased risk of ASCVD, but it is impossible to separate the effects of phytosterol excess from animal fat

reduction in this population [47]. It remains possible that individuals with polymorphisms in the *ABCG5* and *ABCG8* genes that affect phytosterol absorption are at altered risk because of their ability to overabsorb phytosterols [48].

Additional studies on this topic are clearly warranted. These should examine whether increased blood phytosterol concentrations increase ASCVD risk and whether reductions in LDL-C levels by agents such as ezetimibe produce greater than expected reductions in ASCVD risk for the reduction in cholesterol. At the present time, there is not sufficient evidence to advise against phytosterol supplementation to reduce LDL-C levels. Promotion for the use of plant sterols for LDL-C lowering is increasing, but prudence dictates that the dose of phytosterols should not exceed 2 g/day. An additional concern of uncertain significance is the interference with the absorption of certain fat-soluble vitamins [37,49].

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 99–100).

- 1 Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344:1383–1389.
- 2 Downs JR, Clearfield M, Weis S, *et al.* Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. *Air Force/Texas Coronary Atherosclerosis Prevention Study.* *JAMA* 1998; 279:1615–1622.
- 3 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; 26:1126–1133.
- 4 Miettinen TA, Railo M, Lepantalo M, Gylling H. Plant sterols in serum and in atherosclerotic plaques of patients undergoing carotid endarterectomy. *J Am Coll Cardiol* 2005; 45:1794–1801.
- 5 Assmann G, Cullen P, Erbey J, *et al.* Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: results of a nested case–control analysis of the Prospective Cardiovascular Munster (PROCAM) study. *Nutr Metab Cardiovasc Dis* 2006; 16:13–21.
- This describes a prospective study on the elevations of sitosterol and coronary events. The study suggested that elevated sitosterol levels increase cardiovascular risk.
- 6 Sudhop T, Gottwald BM, von Bergmann K. Serum plant sterols as a potential risk factor for coronary heart disease. *Metabolism* 2002; 51:1519–1521.
- 7 Weihrauch JL, Gardner JM. Sterol content of foods of plant origin. *J Am Diet Assoc* 1978; 73:39–47.
- 8 Normen L, Johnsson M, Andersson H, *et al.* Plant sterols in vegetables and fruits commonly consumed in Sweden. *Eur J Nutr* 1999; 38:84–89.
- 9 Kudchodkar BJ, Sodhi HS, Horlick L. Absorption of dietary cholesterol in man. *Metabolism* 1973; 22:155–163.
- 10 Ostlund RE Jr, McGill JB, Zeng CM, *et al.* Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols and phytostanols in humans. *Am J Physiol Endocrinol Metab* 2002; 282:E911–E916.
- 11 Salen G, Ahrens EH Jr, Grundy SM. Metabolism of beta-sitosterol in man. *J Clin Invest* 1970; 49:952–967.
- 12 von Bergmann K, Sudhop T, Lutjohann D. Cholesterol and plant sterol absorption: recent insights. *Am J Cardiol* 2005; 96:10D–14D.
- 13 Garcia-Calvo M, Lisnock J, Bull HG, *et al.* The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *Proc Natl Acad Sci U S A* 2005; 102:8132–8137.

- 14 Altmann SW, Davis HR Jr, Zhu LJ, *et al.* Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science* 2004; 303:1201–1204.
- 15 Heinemann T, Axtmann G, von Bergmann K. Comparison of intestinal absorption of cholesterol with different plant sterols in man. *Eur J Clin Invest* 1993; 23:827–831.
- 16 Gould RG, Jones RJ, LeRoy GV, *et al.* Absorbability of beta-sitosterol in humans. *Metabolism* 1969; 18:652–662.
- 17 Eneroth P, Hellstrom K, Rhyage R. Identification and quantification of neutral faecal steroid studies of human excretion during two dietary regimens. *J Lipid Res* 1964; 5:245–262.
- 18 Eneroth P, Hellstrom K, Rhyage R. Identification of two neutral metabolites of stigmasterol found in human feces, bile acids and sterols I62. *Steroids* 1965; 6:707–720.
- 19 McNamara DJ, Proia A, Miettinen TA. Thin-layer and gas-liquid chromatographic identification of neutral steroids in human and rat feces. *J Lipid Res* 1981; 22:474–484.
- 20 Bhattacharyya AK, Connor WE. Beta-sitosterolemia and xanthomatosis A newly described lipid storage disease in two sisters. *J Clin Invest* 1974; 53:1033–1043.
- 21 Salen G, Shefer S, Nguyen L, *et al.* Sitosterolemia. *J Lipid Res* 1992; 33:945–955.
- 22 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; 102:1041–1044.
- 23 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.
- 24 Salen G, Tint GS, Shefer S, *et al.* Increased sitosterol absorption is offset by rapid elimination to prevent accumulation in heterozygotes with sitosterolemia. *Arterioscler Thromb* 1992; 12:563–568.
- 25 Hidaka H, Nakamura T, Aoki T, *et al.* Increased plasma plant sterol levels in heterozygotes with sitosterolemia and xanthomatosis. *J Lipid Res* 1990; 31:881–888.
- 26 Cobb MM, Salen G, Tint GS, *et al.* Sitosterolemia: opposing effects of cholestyramine and lovastatin on plasma sterol levels in a homozygous girl and her heterozygous father. *Metabolism* 1996; 45:673–679.
- 27 Nguyen LB, Cobb M, Shefer S, *et al.* Regulation of cholesterol biosynthesis in sitosterolemia: effects of lovastatin, cholestyramine, and dietary sterol restriction. *J Lipid Res* 1991; 32:1941–1948.
- 28 Jurado J, Seip R, Thompson PD. Effectiveness of ezetimibe in clinical practice. *Am J Cardiol* 2004; 93:641–643.
- 29 Salen G, von Bergmann K, Lutjohann D, *et al.* Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation* 2004; 109:966–971.
- 30 Miettinen TA, Gylling H, Lindbohm N, *et al.* Serum noncholesterol sterols during inhibition of cholesterol synthesis by statins. *J Lab Clin Med* 2003; 141:131–137.
- 31 Nguyen LB, Salen G, Shefer S, *et al.* Deficient ileal 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in sitosterolemia: sitosterol is not a feedback inhibitor of intestinal cholesterol biosynthesis. *Metabolism* 1994; 43:855–859.
- 32 Peterson DW. Effect of soybean sterols in the diet on plasma and liver cholesterol in chicks. *Proc Soc Exp Biol Med* 1951; 78:143–147.
- 33 Thompson GR, Grundy SM. History and development of plant sterol and stanol esters for cholesterol-lowering purposes. *Am J Cardiol* 2005; 96:3D–9D.
- 34 Grundy SM. Stanol esters as a component of maximal dietary therapy in the National Cholesterol Education Program Adult Treatment Panel III report. *Am J Cardiol* 2005; 96:47D–50D.
- 35 Blair SN, Capuzzi DM, Gottlieb SO, *et al.* Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. *Am J Cardiol* 2000; 86:46–52.
- 36 Miettinen TA, Puska P, Gylling H, *et al.* Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *N Engl J Med* 1995; 333:1308–1312.
- 37 Katan MB, Grundy SM, Jones P, *et al.* Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clin Proc* 2003; 78:965–978.
- 38 Moghadasian MH, McManus BM, Pritchard PH, Frohlich JJ. 'Tall oil'-derived phytosterols reduce atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 1997; 17:119–126.
- 39 Moghadasian MH, McManus BM, Godin DV, *et al.* Proatherogenic and antiatherogenic effects of probucol and phytosterols in apolipoprotein E-deficient mice: possible mechanisms of action. *Circulation* 1999; 99:1733–1739.
- 40 Moghadasian MH, Godin DV, McManus BM, Frohlich JJ. Lack of regression of atherosclerotic lesions in phytosterol-treated apo E-deficient mice. *Life Sci* 1999; 64:1029–1036.
- 41 Moghadasian MH. Dietary phytosterols reduce cyclosporine-induced hypercholesterolemia in apolipoprotein E-knockout mice. *Transplantation* 2006; 81:207–213.
- 42 Plat J, Beugels I, Gijbels MJ, *et al.* Plant sterol or stanol esters, alone or in combination with atorvastatin retard lesion formation in heterozygous LDL receptor deficient mice independent of changes in serum plant sterols. *J Lipid Res* 2006; Sep 6 [Epub ahead of print].
- This was an excellent animal study on plant sterols and atherogenicity exploring atorvastatin induced elevations of phytosterols and atherosclerosis in mice.
- 43 Glueck CJ, Speirs J, Tracy T, *et al.* Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol, and premature coronary heart disease in hyperphytosterolemic probands and their first-degree relatives. *Metabolism* 1991; 40:842–848.
- 44 Sutherland WHF, Williams MJA. Association of plasma non cholesterol sterols with severity of coronary artery disease. *Nutr Metab Cardiovasc Dis* 1998; 8:386–391.
- 45 Rajaratnam RA, Gylling H, Miettinen TA. Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. *J Am Coll Cardiol* 2000; 35:1185–1191.
- 46 Wilund KR, Yu L, Xu F, *et al.* No association between plasma levels of plant sterols and atherosclerosis in mice and men. *Arterioscler Thromb Vasc Biol* 2004; 24:2326–2332.
- 47 Vuoristo M, Miettinen TA. Absorption, metabolism, and serum concentrations of cholesterol in vegetarians: effects of cholesterol feeding. *Am J Clin Nutr* 1994; 59:1325–1331.
- 48 Berge KE, von Bergmann K, Lutjohann D, *et al.* Heritability of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. *J Lipid Res* 2002; 43:486–494.
- 49 Devaraj S, Jialal I. The role of dietary supplementation with plant sterols and stanols in the prevention of cardiovascular disease. *Nutr Rev* 2006; 64:348–354.