Annu. Rev. Nutr. 2002. 22:533–49
doi: 10.1146/annurev.nutr.22.020702.075220

PHYTOSTEROLS IN HUMAN NUTRITION

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Key Words sitosterol, sitostanol, stanols, diet, cholesterol, lipoproteins, LDL

■ **Abstract** Phytosterols are cholesterol-like molecules found in all plant foods, with the highest concentrations occurring in vegetable oils. They are absorbed only in trace amounts but inhibit the absorption of intestinal cholesterol including recirculating endogenous biliary cholesterol, a key step in cholesterol elimination. Natural dietary intake varies from about 167–437 mg/day. Attempts to measure biological effects in feeding studies have been impeded by limited solubility in both water and fat. Esterification of phytosterols with long-chain fatty acids increases fat solubility by 10-fold and allows delivery of several grams daily in fatty foods such as margarine. A dose of 2 g/day as the ester reduces low density lipoprotein cholesterol by 10%, and little difference is observed between Δ^5-sterols and 5α-reduced sterols (stanols). Phytosterols can also be dispersed in water after emulsification with lecithin and reduce cholesterol absorption when added to nonfat foods. In contrast to these supplementation studies, much less is known about the effect of low phytosterol levels in the natural diet. However, reduction of cholesterol absorption can be measured at a dose of only 150 mg during otherwise sterol-free test meals, suggesting that natural food phytosterols may be clinically important. Current literature suggests that phytosterols are safe when added to the diet, and measured absorption and plasma levels are very small. Increasing the aggregate amount of phytosterols consumed in a variety of foods may be an important way of reducing population cholesterol levels and preventing coronary heart disease.

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STRUCTURE AND NOMENCLATURE

Phytosterols are plant-derived sterols that are structurally similar and functionally analogous to cholesterol in vertebrate animals. The chemical structure of common phytosterols is shown in Figures 1 and 2. In many foods sitosterol is the most

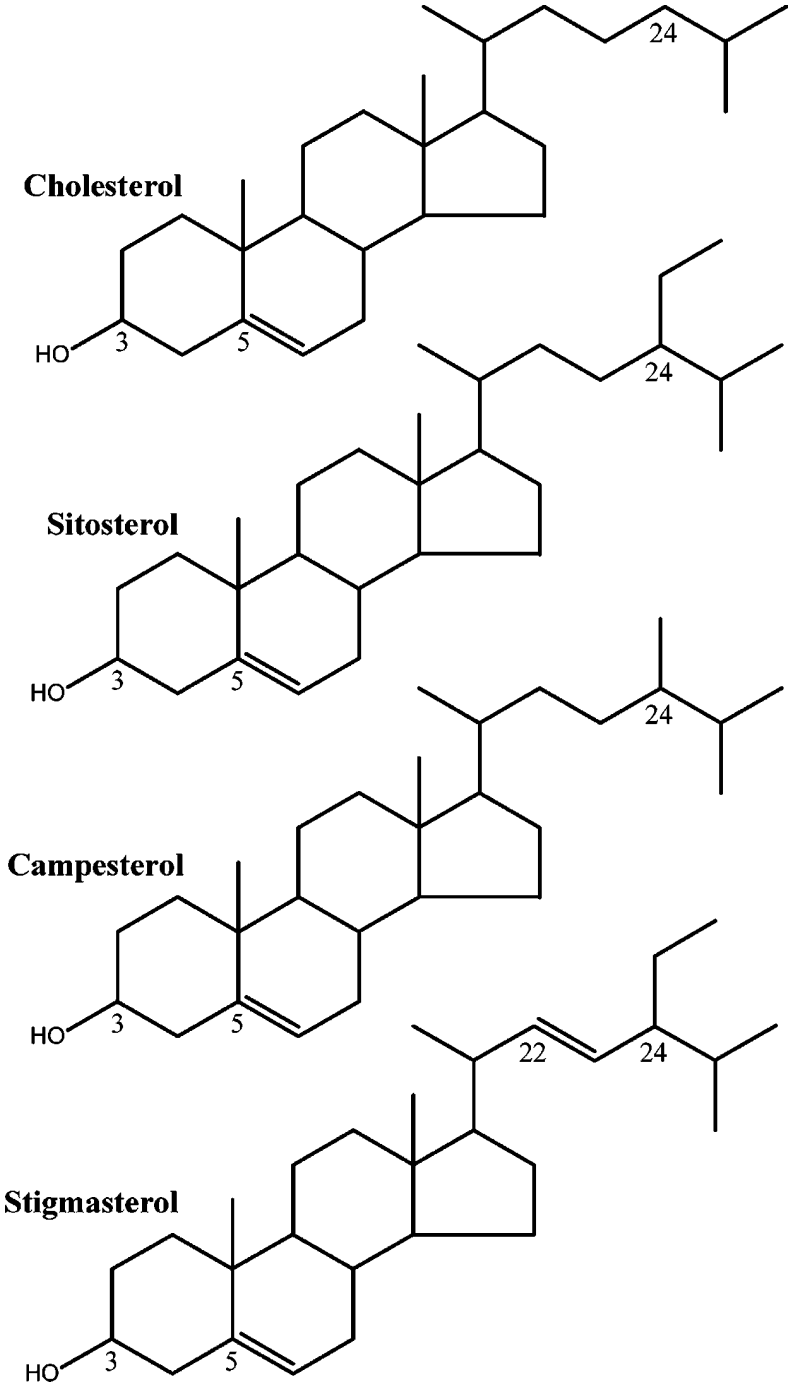

Figure 1 Cholesterol and common Δ^5-phytosterols.

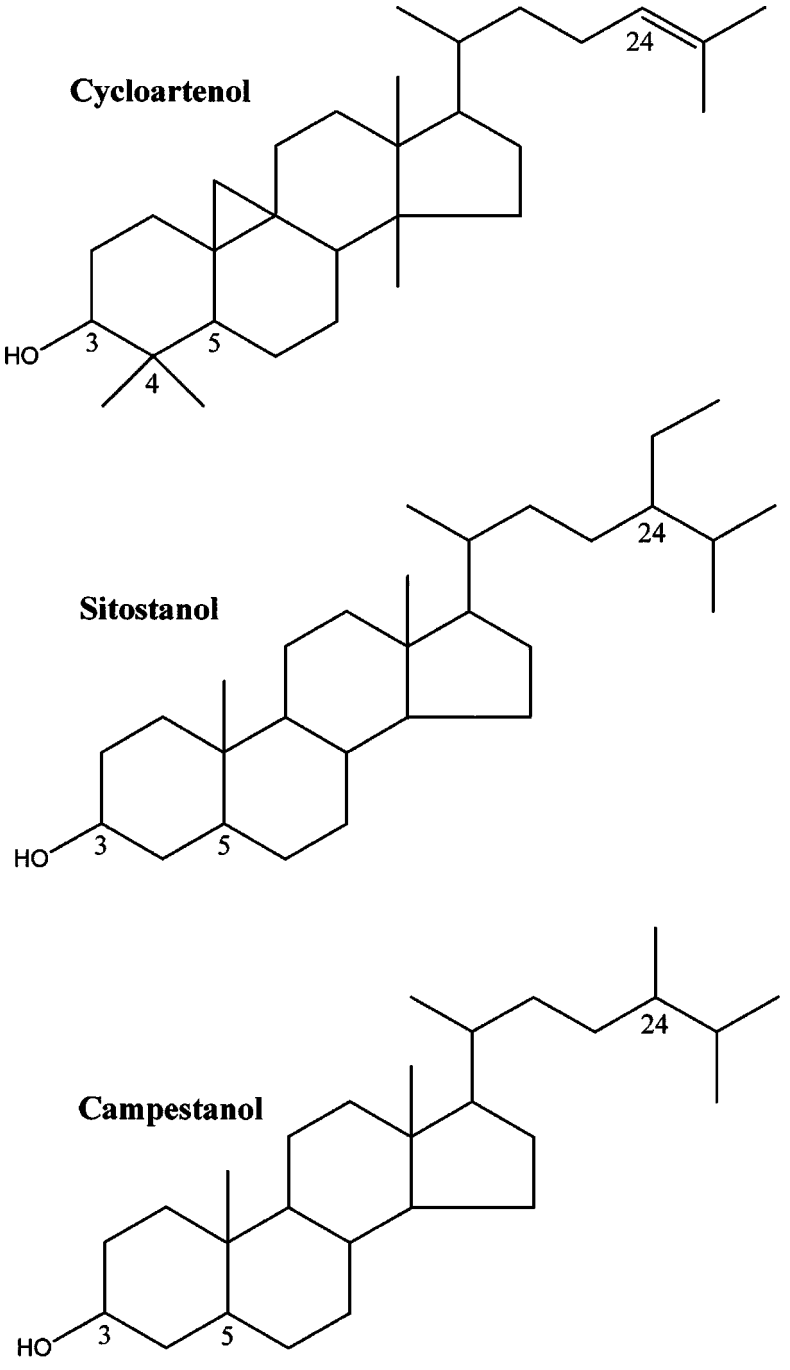

Figure 2 Phytostanols with 5α configuration.

abundant form, followed by campesterol and stigmasterol. Many minor sterols have been reported.

The term "sterol" is traditionally applied by chemists to a substance with the fused cyclopentanophenanthrine ring structure of cholesterol plus an alcohol moiety and, in this sense, all the compounds in Figures 1 and 2 are sterols. In this review the term "phytosterols" is meant to be all-inclusive. However, recent convention among nutritionists has divided phytosterols into the two categories of "sterols" or "Δ^5-sterols," indicating a double bond at position 5, and "stanols," indicating 5α-reduction of that double bond (39). These classes are reflected in the common names of the compounds. For example, sitosterol is structurally identical to sitostanol except for the double bond at position 5, and campesterol bears the same relationship to campestanol. Reduction of the double bond to give a β-hydrogen at position 5 (facing upward from the ring system) is a common intestinal bacterial degradation product of cholesterol and plant sterols but is not generally found in foods (49, 50).

Sterol biosynthesis and metabolism are quite different in plants and animals (17). Cholesterol is synthesized from acetate through the straight-chain compound squalene and then cyclized to form many intermediate precursor sterols, but the final product is most often nearly pure cholesterol, and only trace amounts of related precursors are found in humans (53). In plant cells phytosterols are also synthesized from acetate subunits through squalene, but the first cyclized product after squalene is the unique plant sterol cycloarterol (Figure 2). Some foods, such as rice, contain cycloartenol or related compounds in significant amounts (66). Plants then produce a variety of final sterol products, and each species has a characteristic distribution of sterols that has sometimes been used as a chemical fingerprint for identification of food product sources. Although modifications to the sterol nucleus occur, the most common plant sterols have a nucleus that is identical to cholesterol, with changes occurring in the side chain. In foods three compounds account for most of the phytosterol mass: sitosterol (24-ethylcholesterol), campesterol (24-methylcholesterol), and stigmasterol (Δ^{22}-24-ethylcholesterol). The structural similarity to cholesterol is striking, and it is difficult to separate cholesterol from phytosterols or different phytosterols from each other by physical methods unless powerful techniques such as high pressure liquid chromatography or gas chromatography involving thousands of theoretical plates are employed.

The complexity of phytosterols is increased further by steric asymmetry. Addition of alkyl groups in the side chain results in the substituted carbon becoming asymmetric. For example, soybeans contain two 24-methylcholesterol isomers, campesterol (24-α-configuration) and dihydrobrassicasterol (24-β-configuration) (56). Generally the α-configuration is more common in higher plants, whereas the β-configuration predominates in algae (17). Stereochemistry has not generally been considered in human and animal studies of phytosterols.

In animals most of the total body cholesterol is free, with a relatively small aggregate amount present as long-chain fatty acyl esters in plasma lipoproteins and

specialized cells. Plants have a more diverse variety of derivatives at the 3-position (Figure 3). Long-chain fatty acyl esters are present in most plants and constitute over 50% of the total sterol in foods such as corn oil (40). Ferrulate esters are found in appreciable quantity in many foods (58). Glycosylation occurs more actively in plants than in animals, and glycosylated phytosterols (Figure 3, *bottom*) are a minor component of most foods. However, the amount is sometimes appreciable, and glycosides constitute 82% of potato phytosterols (38). Some glycolipids are also acylated (R group in Figure 3). It should be emphasized that glycosidic linkages are not cleaved by the alkaline conditions used to hydrolyze sterol esters but rather require acidic hydrolysis. Phytosterol glycosides are therefore not measured by common procedures for phytosterol quantitation, and future techniques need to include both alkaline and acidic hydroylsis conditions.

OCCURRENCE IN FOODS

All vegetable foods contain appreciable quantities of phytosterols, but current food databases do not have comprehensive estimates of phytosterol content. Thus, phytosterols in test diets cannot be routinely calculated. Table 1 gives values for total phytosterol content of representative foods (79). It should be noted that glycosylated phytosterols (generally small) are not included. The most concentrated source of phytosterols is vegetable oil. A person consuming 30 g/day of corn oil would receive 286 mg, an amount that has been shown to be bioactive in reducing cholesterol absorption (58) (see below). An exception is palm oil, which is deficient in phytosterols after refining for western markets. Smaller unit amounts are found in nuts, breads, and whole vegetables, but these items also have larger portion sizes. Except for highly refined carbohydrates and animal products, nearly all foods contribute appreciably to phytosterol intake. Estimates of daily phytosterol consumption range from 167–437 mg/day in various populations, approximately the same as cholesterol intake (1, 11, 30, 51, 55).

Although not studied as much as Δ^5-sterols, stanols are also found in the diet. Stanols have been reported in unhydrogenated vegetable oils and cereals (15, 46, 72). An estimate of stanol consumption can be obtained from fecal analyses because bacterial conversion of Δ^5-sterols in the large bowel results in 5β-reduced rather than 5α-reduced metabolites (50). In subjects not receiving stanol treatment, stanol excretion has been reported as 24–29 mg/day (13, 21). This suggests that stanols may comprise about 10% of dietary phytosterol intake.

ABSORPTION AND SAFETY

The absorption of cholesterol is $56.2 \pm 12.1\%$ in normal subjects during test meals (8). However, phytosterols traditionally have been considered to be nonabsorbable. In humans consuming solid food diets more than 90% of sitosterol is recovered in the stool (41) and sitosterol has been used as a nonabsorbable recovery standard in measurements of cholesterol balance and absorption (19, 75). However, a small

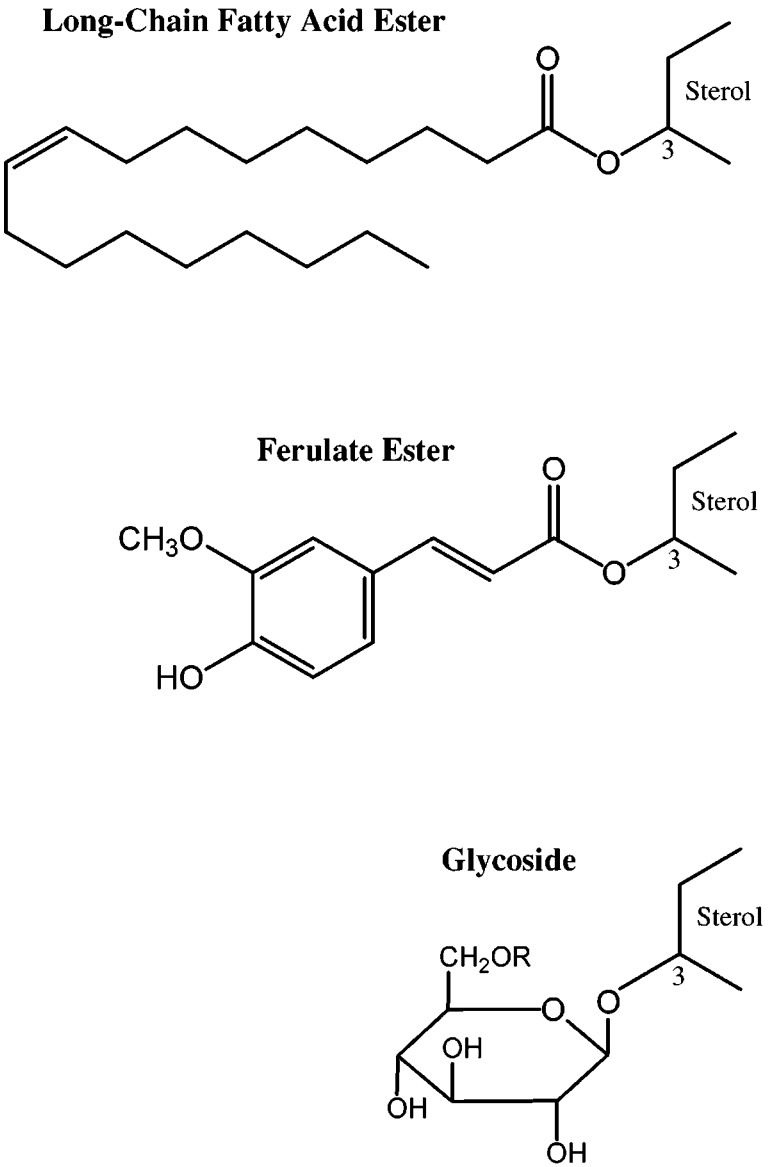

Figure 3 Modifications of the 3-β-hydroxyl group. R indicates an acyl group present in some phytosterol glycosides.

TABLE 1 Phytosterol contents (data from Reference 79)

Food	Phytosterols (mg/100 g edible portion)
Corn oil	952
Sunflower oil	725
Safflower oil	444
Soybean oil	221
Olive oil	176
Almonds	143
Beans	76
Corn	70
Wheat	69
Palm oil	49
Lettuce	38
Banana	16
Apple	12
Tomato	7

but definite absorption occurs. Several studies using radioactive sitosterol have reported that absorption is 0.6–7.5% of the administered amount (45, 67, 70). Percentage absorption varied inversely with the dose, suggesting that a limitation in absorption exists at high intake levels (67). Campesterol absorption has been measured at 9.6% by losses during intestinal intubation (27) and 16% from stool losses determined by mass spectrometry (45). Campestanol was reported to be 12.5% absorbed by losses during intubation (27) and 5.5% absorbed by radioactive assay of plasma (71). The most desirable conditions for measuring phytosterol absorption involve the use of serum samples to establish unequivocal absorption into the systemic circulation rather than just the intestinal mucosa and the use of mass spectrometry for definitive identification of labeled tracers. Recent work using these methods reports the following generally lower values for percent absorption: sitosterol, 0.51%; campesterol, 1.9%; sitostanol, 0.04; and campestanol, 0.16% (57). Taken together, these studies show that phytosterol absorption is quite low, particularly for 5α-stanols, which are absorbed with about 10% of the efficiency of the corresponding Δ^5-sterols.

In humans serum, levels of sitosterol and campesterol are only 0.1–0.14% of the cholesterol concentration (53). When dietary Δ^5-sterols were supplemented at 2–3 g/day, the serum sitosterol and campesterol levels increased by 34–73%, with most values remaining in the normal range (25, 80). Stanols fed in the same dose actually reduced serum Δ^5-sterol levels by 17–36%, presumably by inhibiting

absorption (25, 80). Baseline serum stanols were difficult to measure, with levels estimated at 0.003–0.004% of cholesterol (25). Serum campestanol and sitostanol were unchanged after Δ^5-sterol feeding but increased 200–275% after supplementation with 2 g/day of mixed stanols. However, the absolute levels remained exceedingly small and were less than 0.02% of cholesterol.

Plasma levels of fat-soluble vitamins and other vegetable-derived compounds have been studied following phytosterol feeding because of concern about the possibility of reduced absorption. Because these lipophilic materials are carried in plasma lipoproteins, most studies have normalized levels to plasma lipids or cholesterol. After this procedure serum α- and β-carotene have been reported to be reduced by 11–22% (23, 29), although other studies find only a small reduction without statistical significance (26, 62). However, a correlation has been noted between estimated reduction in cholesterol absorption and reduction of circulating carotenes and lycopene (62). The most affected compounds were the nonpolar hydrocarbons α-carotene, β-carotene, and lycopene. Serum concentrations of 25-hydroxyvitamin D, retinol, and vitamin K were not reduced in the same studies in which carotenoids were affected (23, 29). The significance of reduced serum carotene is not known, but supplementation with β-carotene does not appear to protect against coronary heart disease (9).

One potential safety concern over increases in population exposure to phytosterols is the rare inherited disorder phytosterolemia. This condition is caused by mutations in the ATP-binding cassette proteins ABCG5 and ABCG8, which are expressed in intestine and liver (5). Although increased absorption of phytosterols occurs in this illness and is an important clinical marker, it is likely that abnormal handling of cholesterol contributes most strongly to the resulting atherosclerosis. For example, in a severely affected sitosterolemia homozygote who expired of atherosclerosis, cholesterol accounted for 82% of the plasma, tissue, and atheroma sterol (68). In 14 homozygotes the total plasma sterol concentration was 320 mg/dl, with cholesterol accounting for 81% of the total (69). Heterozygotes appear to be affected much less than homozygotes, with values for plasma phytosterol levels and absorption only modestly elevated (71) and compensated for by an ability to rapidly excrete the absorbed phytosterols (70). It is not clear what effect phytosterol feeding might have on sitosterolemic subjects. In two patients the administration of 1.5 g/day of sitostanol reduced the absorption of both cholesterol and other plant sterols, increased their excretion and lowered plasma levels of all sterols except sitostanol, which remained constant (45). Thus, it is possible that phytosterol feeding might be of benefit in sitosterolemia under some circumstances, but more work is needed in this area.

LOW DENSITY LIPOPROTEIN LOWERING

Over 40 years ago phytosterols were found to lower serum cholesterol in animals and humans (60, 65). Total and low density lipoprotein (LDL) cholesterol are reduced without consistent effects on high density lipoprotein cholesterol and

triglycerides. However, several well-controlled clinical trials have reported that cholesterol lowering was either variable between patients (43) or absent altogether even with large doses (2, 14). In hindsight it appears likely that these difficulties were caused by reduced bioavailability of the preparations used. Both the slow rate of dissolution and low ultimate aqueous and organic solubility appear to contribute. Purified phytosterols form highly stable crystals (the form in which they are usually commercially supplied) that may require several days or even weeks to dissolve in bile salt solutions (3, 59). Consistent LDL lowering has been reported in studies in which macrocrystals were not used but phytosterols were dissolved in oil (52) or egg fat (47), emulsified in aqueous medium with triglycerol monooleate (20) or lecithin (59), or finely micronized and mixed with fatty foods (36, 37). Direct dissolution of free phytosterols in fat is not very efficient because the solubility in triglyceride is only about 1–2%, but after esterification with long-chain fatty acids the solubility increases to 10–20% (47, 48, 77). The margarines Benecol® and Take Control® contain phytosterol esters to increase the amount of phytosterol in the product relative to triglyceride.

When properly solubilized phytosterols are employed, LDL cholesterol is consistently reduced in long-term clinical feeding studies. Figure 4, derived from 26 studies with 36 treatment arms, summarizes the percentage LDL lowering that can be achieved compared with a control group (39, 42). A measurable effect was seen with 900 mg/day, and reduction was nearly maximum (9.6% lowering) at a dose

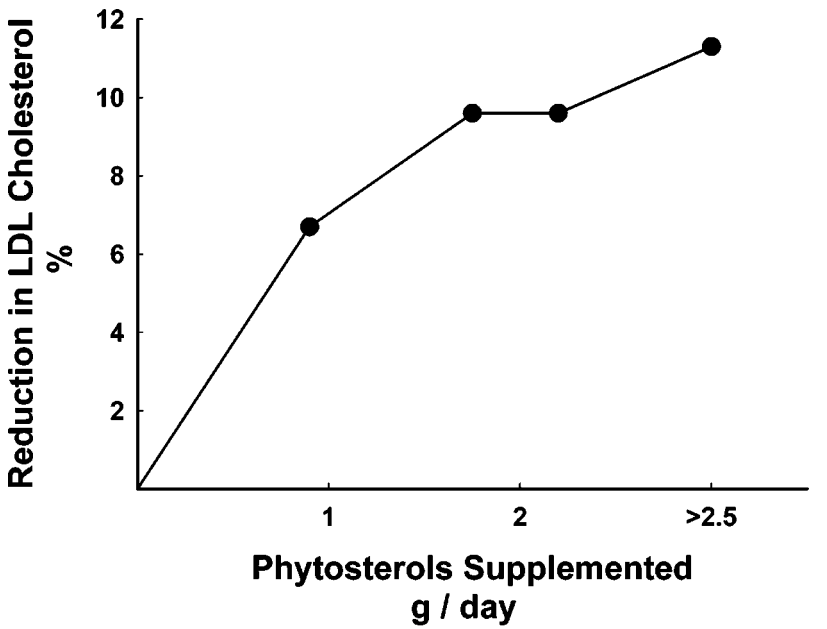

Figure 4 Reduction in low density lipoprotein cholesterol compared with controls in 26 studies of Δ^5-sterol esters and stanol esters (data from Reference 39).

of about 2 g/day, that recommended by the U.S. National Cholesterol Education Program for lifestyle changes to lower serum cholesterol (16). At the highest doses the reduction was 11.3%. Phytosterols are often given 2–3 times/day, but a recent study showed similar LDL lowering when the same dose was given once daily (64).

Considered from both the viewpoint of an individual patient as well as the population, reductions of about 10% in LDL are significant and could be associated with a similar reduction in near term coronary heart disease risk when used in primary prevention (81). Phytosterols are also effective when combined with statin drugs and could be useful in secondary prevention of heart disease when greater targeted reductions in LDL cholesterol are needed (7). The combination of phytosterols, which increase cholesterol elimination, and statins, which reduce cholesterol biosynthesis, is attractive. For comparison, the new cholesterol absorption–blocking drug ezetimibe reduced LDL cholesterol by 18.5% as monotherapy in clinical trials (4).

There is little evidence for clinically significant differences in the effectiveness of common phytosterols on cholesterol lowering. One well-controlled study, which used purified phytosterols and was performed in rats, found no differences in effects on cholesterol absorption between esterified and nonesterified Δ^5-sterols or between purified sitosterol oleate, stigmasterol oleate, and campesterol oleate (48). Another study found that purified sitostanol was slightly superior to sitosterol in rats but that both were quite effective and nearly equal when the baseline serum cholesterol level was highest (73). Intubation studies in humans reported that sitosterol infusion lowered cholesterol absorption by 50%, compared with 85% for sitostanol (28). However, the potential advantage of stanols over Δ^5-sterols could not be statistically confirmed during chronic feeding studies in humans using LDL lowering as an endpoint. Head-to-head comparison of Δ^5-sterols and stanols in controlled trials found them to be equally effective (35, 54, 80). Stanols synthesized from vegetable oil Δ^5-sterols by chemical reduction and containing only 68% sitostanol gave the same reduction in LDL cholesterol as wood-derived stanols containing 92% sitostanol (23, 61, 62).

4,4-Dimethylsterols found in foods such as sheanut and rice bran may be an exception to the rule of general efficacy of phytosterols. Sheanut sterols (triterpene alcohols) do not lower LDL (78, 80). Relatively pure rice bran 4,4-dimethylsterols have been reported to be ineffective (80), whereas rice bran sterols containing about half 4,4-desmethysterols (such as sitosterol) have a small effect consistent with that expected from 4,4-desmethylsterols alone (78). The available evidence supports either little or no activity of 4,4-dimethylsterols on LDL levels in humans.

IMPORTANCE IN THE NATURAL DIET

All of the studies of LDL lowering by phytosterols cited above have a fundamental flaw: The effect of supplemented phytosterols is considered only with respect to the phytosterol-containing baseline diet and not with respect to a phytosterol-free diet. Whereas this is appropriate for deciding whether supplementation of typical

diets is effective, it does not answer important questions about the potential role of lower levels of food phytosterols. Moreover, this bias tends to underestimate the effectiveness of phytosterols by making the tacit assumption that no baseline diet effect exists. When we say that phytosterols reduce LDL cholesterol by 10%, we mean that additional phytosterols can reduce LDL by 10% over the unknown basal effect of dietary phytosterols.

Very few clinical diet studies have carefully controlled and reduced baseline levels of phytosterols because of the inherent difficulty of doing so: Solid-food diets generally contain plant materials, almost all of which have significant quantities of phytosterols. A phytosterol-free diet is a liquid formula diet that uses milk protein, refined sugar, and sterol-free fats and oils. Although animal fats are phytosterol-free, vegetable fats are rich in phytosterols (Table 1) and very difficult to purify (or to resynthesize from purified fatty acids) on a kilogram scale. Recent work comparing purified corn oil triglyceride with or without corn oil phytosterols to commercial corn oil in single test meals reported that cholesterol absorption increased 38% when corn oil was purified (58). The effect was attributed to natural corn oil phytosterols because adding them back to the purified oil gave results similar to the original commercial oil. As little as 150 mg of corn oil phytosterols had a measurable effect on cholesterol absorption. This suggests that consumption of vegetable oils may reduce LDL through their phytosterol content as well as through the effects of fatty acids.

MECHANISM OF ACTION

The original publication relating phytosterol feeding to changes in serum cholesterol levels noted that dietary cholesterol absorption appeared to be inhibited and the response to a high-cholesterol diet attenuated (60). This mechanism has withstood the test of time and scientific scrutiny, but much remains to be learned about its details. The effect of phytosterols on cholesterol absorption is incomplete. In contrast to the drug ezetimibe, which can reduce cholesterol absorption by more than 90% in animals (10, 76), cholesterol absorption is reduced by about 30–50% at maximum doses of phytosterols (43, 48, 59). Cholesterol absorption is a very important physiological mechanism that is not limited to dietary cholesterol intake. Both dietary cholesterol (∼300 mg/day) and recirculating biliary cholesterol (∼1000 mg/day) mix in the intestine and are partially absorbed (18). Failure to reabsorb intestinal cholesterol is the principal means of cholesterol elimination from the body.

Because phytosterols are not systemically absorbed, research has focused on actions that occur within the intestinal lumen. In vitro and in vivo studies of animals showed that phytosterols compete with and displace cholesterol from bile salt/phospholipid micelles, the form from which cholesterol absorption occurs (32–34). A careful analysis of the physical chemistry involved showed that sitosterol has increased affinity for biliary micelles compared with cholesterol, so sitosterol uptake by micelles is energetically favored (3). The effect occurred in micelles

containing the trihydroxy bile salt taurocholate and not with the dihydroxy bile salt glycodeoxycholate, suggesting that the distribution of bile salts present in the intestine may be important in determining the effectiveness of phytosterols in reducing cholesterol absorption. The notion that lack of complete solubilization of cholesterol in bile salt/phospholipid micelles can limit absorption is supported by studies in which cholesterol and sitosterol were presolubilized in an excess of such micelles and then perfused over many hours into the small bowel of animals with biliary diversion; no reduction in cholesterol absorption was observed when phytosterols were included in the perfusate (34). Further evidence of the importance of micellar solubility is the finding that the absorbability of different sterols is directly related to their equilibrium micellar concentration (3).

Even though evidence for intraluminal phytosterol action is compelling, it is possible that phytosterols may also work within the enterocyte. It is established that phytosterols are retained in the intestinal epithelium after oral ingestion (6, 31). Thus, the idea that phytosterols are unabsorbable is not correct when viewed with respect to the intestine itself. It is likely that phytosterols are incorporated into the plasma membrane of the enterocyte and possibly internalized as well. The latter is suggested by the finding in phytosterolemia that systemic absorption of phytosterols is associated with deficiency of the ABCG5 and ABCG8 proteins that appear to remove sterols from cells (5). In studies in which the transport of cholesterol and sitosterol directly to intact intestinal mucosa or mucosal cells has been measured, the transfer of sitosterol has been significant, but with a rate that is 1/3 to 1/5 that of cholesterol (12, 31). Competition of sitosterol with cholesterol for uptake into intestinal epithelium can be demonstrated at high sterol concentrations (34). More work is needed to determine the extent of interaction of phytosterols with cholesterol within the enterocyte.

Inhibition of cholesterol absorption by the above mechanisms produces a state of relative cholesterol deficiency that is followed by upregulation of cholesterol biosynthesis and LDL receptor activity (44). After chronic phytosterol feeding whole body cholesterol biosynthesis measured by incorporation of deuterated water increased by 38–53% (35) and cholesterol precursor sterol concentration in the serum, an indicator of cholesterol biosynthetic rate, rose 10–46% (24). LDL receptor expression measured at the RNA and protein level increased 25–43% and changes in LDL levels correlated with LDL receptor expression in peripheral cells (63). At the level of the liver the LDL production rate was reduced by 20% and the plasma concentration of dense LDL was reduced by 22% with light LDL remaining unchanged (22). The measured fractional rate of LDL apo B catabolism was not changed, probably owing to the qualitative change in LDL.

FUTURE DIRECTIONS

The past 10 years have seen the early academic studies of phytosterol metabolism reduced to practical commercial products that lower LDL cholesterol with minimal risk. Although more work needs to be done on the safety of phytosterols, it is likely

the population risk of heart disease can be reduced appreciably over time by the use of more phytosterols in foods and supplements. Up to this time attention has been focused principally on the addition of phytosterol esters to fatty foods. This needs to be complemented by development of biologically active phytosterol preparations that contain minimal fat and are capable of being added to a variety of foods including nonfat foods.

However, perhaps the most important future work needs to be focused on the potential role of the lower levels of phytosterols in the natural diet and standard foods. Food databases need to include phytosterol levels to allow dietary consumption to be calculated. Clinical trials need to be done to evaluate the role of phytosterols in vegetable oils and other foods with respect to cholesterol lowering in which both the phytosterol content and the fatty acid composition of the diet are measured and controlled. Finally, our manufacturing processes need to emphasize the retention of phytosterols in foods. Phytosterols from food streams currently have economic value, but it makes little societal sense for us to remove phytosterols from some foods in order to place them in others. Refining of vegetable oils can reduce the phytosterol content substantially, taking out most of the phytosterols and carotenoids from steam-refined palm oil (74) and making substantial reductions in other oils (40, 79). With cooperation between the food industry and academia phytosterols may prove to be a useful tool to reduce cholesterol levels in large numbers of people with minimum expense and risk.

ACKNOWLEDGMENTS

This work was supported by grant 50420 from the National Institutes of Health. Dr. Ostlund owns an equity interest in Lifeline Technologies, Inc., a producer of phytosterol supplements.

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