

Sitostanol administered in lecithin micelles potently reduces cholesterol absorption in humans¹⁻³

Richard E Ostlund Jr, Curtis A Spilburg, and William F Stenson

ABSTRACT

Background: Phytosterol feeding in human clinical trials has had generally small and inconsistent effects on serum cholesterol concentrations, raising doubts about the importance of phytosterols in natural diets and supplements.

Objective: The hypothesis tested was that the low intestinal bioavailability of purified phytosterols can be increased by formulation with lecithin.

Design: The ability of sitostanol to reduce cholesterol absorption was measured directly by including hexadeuterated cholesterol tracer in a standard test breakfast and measuring plasma tracer concentration 4 and 5 d later by gas chromatography–negative ion mass spectrometry. The tracer amount after a test meal containing sitostanol was compared with that after an identical meal containing placebo. Each subject served as his or her own control and the order of testing was random. Sitostanol was formulated either as a powder or as a sonicated micellar solution with lecithin. A total of 38 single-meal tests were performed in 6 healthy subjects.

Results: Sitostanol powder (1 g) reduced cholesterol absorption by only $11.3 \pm 7.4\%$ ($P = 0.2$), confirming in vitro data showing poor solubility of sitostanol powder in artificial bile. In contrast, sitostanol in lecithin micelles reduced cholesterol absorption by $36.7 \pm 4.2\%$ ($P = 0.003$) at a dose of 700 mg and by $34.4 \pm 5.8\%$ ($P = 0.01$) at a dose of 300 mg.

Conclusions: Sitostanol reduced cholesterol absorption at doses lower than reported previously, but only if presented in lecithin micelles. Properly formulated sitostanol as well as naturally occurring complexes of phytosterol and phospholipid might be therapeutically useful for cholesterol lowering. *Am J Clin Nutr* 1999;70:826–31.

KEY WORDS Phytosterols, sitostanol lecithin, cholesterol absorption, deuterium, spectrum analysis, mass

INTRODUCTION

Phytosterols, plant sterols that are structurally similar to cholesterol, are present in foods and reduce intestinal cholesterol absorption (1, 2). Sitostanol, a 5- α -reduced metabolite of the common plant sterol sitosterol, is particularly effective (3, 4). With the exception of the oral antibiotic neomycin (5), no currently available pharmaceuticals block cholesterol absorption as a primary mechanism of action. Phytosterols, therefore, might complement the widely used statin drugs that inhibit cholesterol

biosynthesis. Reduced cholesterol absorption would promote the fecal excretion of both dietary and endogenous biliary cholesterol; the latter accounts for two-thirds of the intestinal cholesterol load (6, 7). Because phytosterols are poorly absorbed, little or no systemic toxicity is expected (6–8).

Previous clinical trials of cholesterol lowering by phytosterols used large doses and yielded variable results. For example, 3–18 g sitosterol/d was found to reduce plasma cholesterol concentrations by only 5–12% (9). There was marked patient-to-patient variability: some subjects experienced consistent and substantial effects with repeated measurement under metabolic ward conditions whereas others appeared to have no response at all. A review of 8 clinical phytosterol trials reported LDL-cholesterol reductions varying from 7% to 33% (10), but a recent well-controlled study failed to find any LDL reduction when 3 g sitostanol/d was used (11).

Previous studies were hampered by the difficulty of measuring cholesterol absorption and most studies measured only the response of plasma cholesterol to phytosterol feeding over weeks to months. With this approach, it is difficult to compare different phytosterol formulations or doses. In the present study, we measured the reduction in cholesterol absorption by sitostanol directly by using a new technique.

SUBJECTS AND METHODS

Reagents

Sitostanol was obtained from Medical Isotopes, Inc (Pelham, NH). Water was removed as a chloroform:water azeotrope by twice dissolving the sitostanol in chloroform followed by evaporation and lyophilization for 72 h. The final product was ground to

¹From the Division of Endocrinology, Diabetes, and Metabolism and the Division of Gastroenterology, Washington University, St Louis, and Lifeline Technologies, Inc, Chesterfield, MO.

²Supported by NIH grant R01-50420, the Washington University General Clinical Research Center (RR-00036), and the Washington University Mass Spectrometry Resource (RR-00954).

³Address reprint requests to RE Ostlund Jr, Division of Endocrinology, Diabetes, and Metabolism, Box 8127, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110. E-mail: ROstlund@imgate.wustl.edu.

Received September 16, 1998.

Accepted for publication March 25, 1999.

TABLE 1
Characteristics of the study subjects¹

Age (y)	39.2 ± 6.6
BMI (kg/m ²)	25.5 ± 3.9
Cholesterol (mmol/L)	5.15 ± 1.18
Triacylglycerols (mmol/L)	1.90 ± 1.38
HDL cholesterol (mmol/L)	1.18 ± 0.49
Percentage cholesterol absorption (%)	49.0 ± 9.8

¹ $\bar{x} \pm SD$; $n = 2$ women, 4 men. Percentage cholesterol absorption was measured in 5 of the subjects by a dual stable-isotope technique and the results were reported previously (15).

a powder with a mortar and pestle. Soy lecithin containing no phytosterols (General Nutrition, Pittsburgh) was dissolved in chloroform, a small amount of nonlipid was removed by centrifugation at $1000 \times g$ for 10 min at room temperature, and the material was dried and lyophilized for 72 h. [26,26,26,27,27,27 - 2H_6]Cholesterol (hexadeuterated cholesterol) was obtained from Medical Isotopes, Inc. [5,6 - 3H]Sitostanol (1724 GBq/mmol) was purchased from Dupont NEN (Boston) and repurified by thin-layer chromatography in 98:2 chloroform:acetone on silica gel G (Fisher Scientific, Pittsburgh). [1,2 - 3H]Cholesterol (1887 GBq/mmol) was purchased from ARC, Inc (St Louis).

Measurement of changes in cholesterol absorption

In previous studies, we used dual stable-isotope tracers, one given orally and one intravenously, to assess percentage cholesterol absorption in a single test meal by measuring the resulting plasma cholesterol enrichments (12). The intravenous tracer was used as a measure of the effective size of the endogenous cholesterol pools. In the present study, we omitted the intravenous tracer and instead of measuring percentage cholesterol absorption we measured the concentration of the oral cholesterol tracer in plasma cholesterol (mmol deuterated cholesterol/mol natural cholesterol) under different experimental conditions. If the cholesterol pool size is constant, changes in oral cholesterol tracer concentration recorded during different cholesterol-absorption tests reflect changes in the efficiency of intestinal cholesterol absorption. Because the rapidly miscible pool into which absorbed cholesterol enters is $\approx 24\ 000$ mg and has a turnover rate of only 0.13 pools/d (13, 14), it is not likely that single meals would significantly change the metabolic parameters of cholesterol. To ensure that cholesterol absorption was complete, we obtained plasma samples 4 and 5 d after tracer administration even though previous work showed that 3 d is sufficient (12). Repeated measures of percentage cholesterol absorption in the same individuals are consistent; the SD of differences between tests is 2.8% (12). Changes in cholesterol absorption resulting from sitostanol administration were calculated from the plasma cholesterol tracer concentration in tests with and without sitostanol.

Clinical protocols

Six healthy, weight-stable subjects (**Table 1**) not taking prescription medications and without active medical or surgical illnesses underwent a total of 38 cholesterol-absorption tests in 3 independently controlled clinical studies approved by the Washington University Human Studies Committee. All subjects underwent 7 cholesterol-absorption tests except for 1 subject who completed only the first 3 (study 1 below). This individual was excluded from studies 2 and 3 because his baseline serum

triacylglycerol concentration in study 2 exceeded 8 mmol/L. Cholesterol-absorption tests were separated by ≥ 2 wk to allow sufficient time for the concentrations of plasma isotopic cholesterol absorbed in previous tests to stabilize.

For each cholesterol-absorption test, subjects were instructed by a dietitian to consume a weight-maintaining National Cholesterol Education Program Step I diet at home for 8 d. On day 4 of the diet, a standard breakfast prepared by the metabolic kitchen of the Washington University General Clinical Research Center was supplied to the subjects along with a treatment or placebo. The test breakfast consisted of 240 mL orange juice, 240 mL whole milk, 21 g corn flakes, and a 60-g bagel saturated with 2.5 g corn oil containing 40 mg thoroughly dissolved hexadeuterated cholesterol. The test breakfast contained 2130 kJ energy, 74 mg cholesterol (including the tracer), and 1.7 g total dietary fiber. Fasting EDTA-treated plasma samples for measuring cholesterol enrichment were drawn on day 4 immediately before isotope administration (baseline) and again on days 8 and 9. The subjects' diets were not controlled during the week between absorption tests.

Three internally complete clinical studies, consisting of 2 or 3 individual cholesterol-absorption tests, were performed sequentially. The studies were single-blind, the treatment or placebo was given in random order, and individual absorption tests were separated by 2 wk.

Study 1

In study 1, subjects underwent 3 cholesterol-absorption tests (1A, 1B, and 1C) to compare 1000 mg powdered sitostanol, 700 mg micellar sitostanol, and placebo. In test 1A, finely divided sitostanol powder (1000 mg) was given in 2 gelatin capsules (Eli Lilly, Indianapolis) with the test breakfast. Gelatin capsules dissolve in water in < 30 s and are designed to deliver their contents rapidly in the stomach. Subjects also received a placebo drink containing lecithin vesicles without sitostanol prepared as described for test 1B.

Test 1B included sitostanol-lecithin vesicles containing 700 mg sitostanol. Micellar sitostanol was prepared by drying a 3:1 molar ratio of soy lecithin and sitostanol from a chloroform solution and lyophilizing the mixture for 72 h. The solid was mixed with water, sonicated for 30 min, and passed through a 5.0- μ m filter (Acrodisc 4199; Gelman Sciences, Ann Arbor, MI). Sitostanol-lecithin vesicles had a mean diameter of 247 nm and lecithin vesicles had a mean diameter of 205 nm as measured with a Zetasizer calibrated at 250 nm (Malvern Instruments, Ltd, Southborough, MA). The amount of sitostanol given was determined by using the Cholesterol CII enzymatic assay (Wako, Richmond, VA) with sitostanol as a standard. The vesicles were diluted to 60 mL with water and flavored with Crystal Lite (Kraft, Inc, White Plains, NY). Two placebo capsules containing 1000 mg glucose were also given. In test 1C, the breakfast included 2 placebo capsules containing glucose and a placebo drink containing lecithin vesicles without sitostanol.

Study 2

In study 2, subjects underwent 2 cholesterol-absorption tests (2A and 2B) to compare 300 mg sitostanol in lecithin vesicles with placebo. For test 2A, sitostanol-lecithin vesicles containing 300 mg sitostanol were prepared as described for study 1. For test 2B, lecithin vesicles without sitostanol (placebo) were given.

Study 3

In study 3, subjects underwent 2 cholesterol-absorption tests (3A and 3B) to compare 100 mg sitostanol in lecithin vesicles with placebo. For test 3A, sitostanol-lecithin vesicles containing 100 mg sitostanol were prepared as described for study 1. For test 3B, lecithin vesicles without sitostanol (placebo) were given.

Mass spectrometry

Our methods for measuring cholesterol tracers diluted in plasma cholesterol were reported previously (12, 15, 16). All plasma samples were analyzed by negative ion mass spectrometry of pentafluorobenzoyl cholesterol esters. Plasma samples (0.5 mL) were saponified (17) and sterols were extracted into petroleum ether and dried. Toluene (200 μ L) was added first, followed by 20 μ L dry pyridine and 5 μ L pentafluorobenzoyl chloride (Sigma, St Louis), and the mixture was vortex mixed and allowed to stand at room temperature for 10 min. Water was added and the pentafluorobenzoyl esters were extracted, dried, and taken up in toluene at a concentration of 0.125 g/L. One microliter was then injected into a gas chromatograph (model 5890; Hewlett-Packard, Palo Alto, CA), split 1:10, and then separated on an Rtx-200 column (15 m, 0.32-mm internal diameter, 0.5- μ m film thickness, trifluoropropylmethyl polysiloxane; Restek Corporation, Bellefonte, PA) with a temperature program of 240 $^{\circ}$ C for 1 min followed by a 20 $^{\circ}$ C/min rise to 300 $^{\circ}$ C, which was held for 5 min. The effluent was admitted into a mass spec-

trometer (model 5988A; Hewlett-Packard) operating in negative ion chemical ionization mode with methane as the reagent gas at 93 Pa and an ion source temperature reduced to 120 $^{\circ}$ C to lower background noise. Selected ions for cholesterol pentafluorobenzoate at a mass-to-charge ratio (m/z) of 581 ($M+1$)⁻ and m/z 586 ($M+6$)⁻ were monitored. The cholesterol peak area ratio at masses 586/581 was reduced by the ratio in the baseline sample, and the deuterated cholesterol concentration in plasma cholesterol was read from a standard curve of hexadeuterated cholesterol diluted in natural cholesterol and similarly corrected for baseline enrichment. The final results were expressed as mmol hexadeuterated cholesterol/mol natural cholesterol. All samples from a given subject were analyzed in a single assay and the within-assay CV was 1.3%.

In vitro dispersion of sterols

[³H]Sitostanol (1.85 kBq) and 1.2 μ mol natural sitostanol were mixed in chloroform with or without 1.2 μ mol soy lecithin and then dried in 1.5-mL microfuge tubes and lyophilized for 30 min. [³H]Cholesterol and [³H]cholesterol oleate were prepared similarly. Artificial bile was made by placing 8 mmol sodium taurocholate/L and 5 mmol soy lecithin/L in 0.15 mol sodium chloride/L containing 15 mmol sodium phosphate/L, pH 7.4; rotating the solution gently overnight at room temperature; and then passing the solution through a 5- μ m filter. At the start of the experiment, 0.5 mL artificial bile was added to each microfuge tube and the tubes were rotated end-over-end at 8 rpm at 37 $^{\circ}$ C for various times. The tubes were then centrifuged at 17000 \times g for 1 min at room temperature and the supernate removed and counted.

Statistics

The 3 clinical studies were conducted sequentially and each was completed and analyzed for a predetermined endpoint before the next began. Each study was analyzed independently by using the general linear model of SAS (version 6.07; SAS Institute Inc, Cary, NC). For study 1, repeated-measures analysis of variance was used; for studies 2 and 3, paired *t* tests were performed. Means \pm SEMs are given in the text.

RESULTS

Sitostanol was poorly soluble in artificial bile in vitro. As shown in Figure 1A, only 2.2% of sitostanol was soluble after 180 min. This increased slowly to 12.8 \pm 0.7% at 48 h (not shown). In contrast, when an equimolar amount of lecithin was included with the solid sitostanol, 28.9 \pm 2.2% was soluble within 180 min. Lecithin had to be intimately mixed with the sitostanol because the artificial bile contained excess lecithin and was not effective by itself. The results for cholesterol, which were similar, are shown in Figure 1B. The dispersibility of sitostanol, cholesterol, and cholesterol oleate in the presence and absence of lecithin is reported in Table 2. The dispersibility was similar, suggesting that solid sterols as a class are poorly soluble in artificial bile and that solubility can be much improved if the sterol is first associated with lecithin.

Human studies were performed to evaluate a single-meal technique for assessing the action of sitostanol on cholesterol absorption. Previous work showed that oral cholesterol tracers do not appear immediately in plasma but rather that absorption and distribution to the plasma takes \approx 3 d (12). Cholesterol concentrations in plasma 4 and 5 d after tracer administration were not

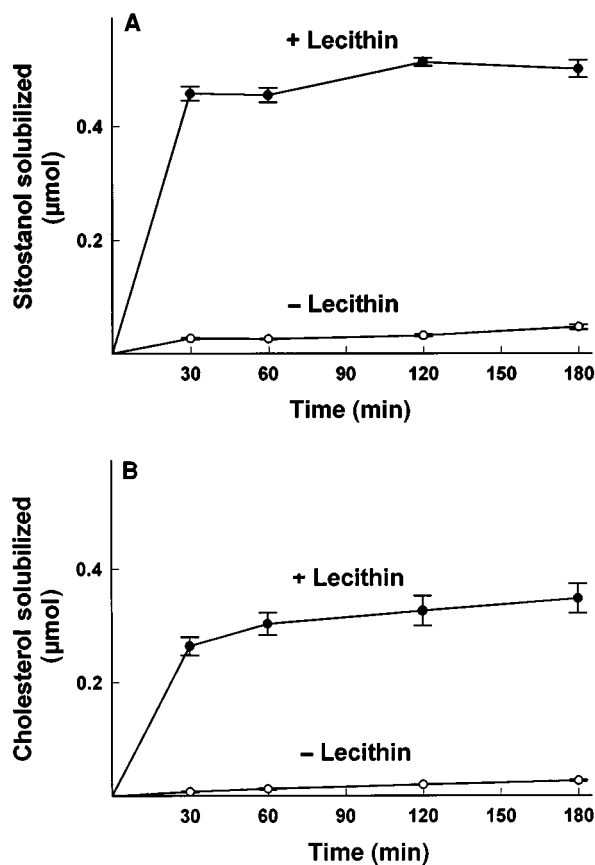


FIGURE 1. Time course of in vitro solubilization of sitostanol (A) and cholesterol (B) in artificial bile. Samples containing 1.2 μ mol sterol \pm 1.2 μ mol soy lecithin were dried and rotated with artificial bile as described in the Methods. Results are means \pm SEMs for triplicate tubes.

TABLE 2
Solubilization of sterols in a micellar bile salt solution¹

	Supernate sterol content
	μmol
Sitostanol	
No lecithin	0.027 ± 0.0015
+ Lecithin	0.458 ± 0.0125
Cholesterol	
No lecithin	0.0072 ± 0.0007
+ Lecithin	0.264 ± 0.016
Cholesterol oleate	
No lecithin	0.0115 ± 0.0003
+ Lecithin	0.416 ± 0.029

¹ $\bar{x} \pm \text{SEM}$; results are for quadruplicate samples.

significantly different and showed an average decline of 7.4% between the 2 d, reflecting slow clearance of absorbed cholesterol from the plasma pool (Table 3). Because both overall and individual results did not differ when calculations were done with samples from days 4 and 5, these data points were averaged for each subject. Shown in Figure 2 are the individual results for study 1 expressed as mmol hexadeuterated cholesterol/mol plasma cholesterol. Sitostanol powder had an inconsistent effect: in 2 subjects plasma tracer concentrations increased slightly, in 1 subject they were minimally reduced, and in 3 subjects they were more substantially reduced. In contrast, sitostanol-lecithin vesicles caused a reduction in plasma tracer concentrations in all subjects. The mean concentration of hexadeuterated cholesterol was reduced from 0.442 ± 0.043 mmol/mol in the absence of sitostanol to 0.382 ± 0.028 mmol/mol for solid sitostanol and to 0.274 ± 0.022 mmol/mol for sitostanol vesicles. The percentage reduction in cholesterol absorption compared with placebo was only $11.3 \pm 7.4\%$ (NS) for 1 g sitostanol powder but $36.7 \pm 4.2\%$ ($P = 0.003$) for 700 mg sitostanol in sitostanol-

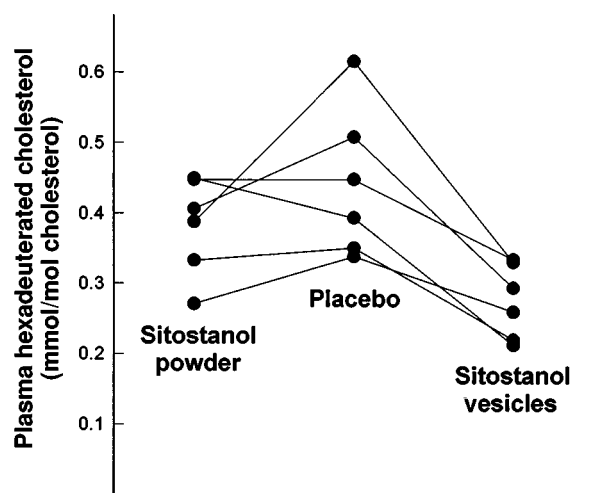


FIGURE 2. Effect of sitostanol on the absorption of hexadeuterated cholesterol in 6 subjects. The mean enrichment of hexadeuterated cholesterol in plasma cholesterol at 4 and 5 d is given for each test meal condition.

lecithin vesicles. This difference between the 2 forms of sitostanol was significant ($P = 0.017$).

Further clinical studies were performed to determine whether a lower dose of sitostanol packaged in lecithin vesicles could reduce cholesterol absorption. In study 2, cholesterol absorption was reduced $34.4 \pm 5.8\%$ by 300 mg sitostanol in vesicles compared with vesicles alone ($P = 0.01$). In study 3, cholesterol absorption was reduced by $5.6 \pm 7.2\%$ by 100 mg sitostanol in vesicles compared with vesicles alone (NS). These data imply that the effective dose of sitostanol lies between 100 and 300 mg and that little additional reduction can be obtained by going to a higher dose. The data from all 3 studies are summarized in Figure 3.

DISCUSSION

Reduced phytosterol bioavailability appears to account in part for the variable results observed in previous cholesterol-lowering clinical trials (9–11). Sterols form highly stable crystals in which the hydrophilic hydroxyl groups are sequestered inside the matrix and are unavailable to solubilizing fluids (18). As a result, sitostanol powder dissolves slowly in bile salt micelles, requiring several days to reach equilibrium (19). The clinical experiments that showed the largest effects used dispersed phytosterols such as those dissolved in triglycerol monooleate (20), cooked in eggs (21), or esterified and dissolved in vegetable oil (22).

Few studies have directly compared different phytosterol formulations in humans. We report here a new method for assessing the effects of phytosterols on cholesterol absorption that entails single-meal tests repeated at biweekly intervals. This is a convenient system in which the effects of phytosterols and other inhibitors of cholesterol absorption can be determined quickly under controlled dietary conditions without administering radioactivity or collecting stool samples. This method complements 8 previously reported methods for measuring cholesterol absorption (23) and differs from them principally by focusing on differences in cholesterol absorption due to a treatment rather than on absolute or percentage cholesterol absorption values. The change in the percentage of cholesterol tracer absorbed during the test meal was proportional to the change in the mass of cholesterol absorbed because the test meal was constant and there was no intervention that would have changed biliary cholesterol secretion. Negative ion mass spectrometry is sensitive enough to require only 40 mg cholesterol tracer per test so that the results are applicable to recommended low-cholesterol diets.

We found that sitostanol reduced cholesterol absorption in our subjects, but only if formulated with lecithin. Pure sitostanol powder (1 g) had no significant overall effect on cholesterol absorption (Figures 2 and 3). This may explain the lack of reduction

TABLE 3

Concentration of hexadeuterated tracer in plasma cholesterol 4 and 5 d after oral administration of hexadeuterated cholesterol and sitostanol or placebo¹

	Day 4	Day 5
	mmol/mol	
Placebo	0.453 ± 0.039	0.430 ± 0.048
Sitostanol powder (1000 mg)	0.410 ± 0.031	0.355 ± 0.028
Sitostanol vesicles (700 mg)	0.279 ± 0.028	0.269 ± 0.020

¹ $\bar{x} \pm \text{SEM}$; $n = 6$. Unit is mmol hexadeuterated cholesterol/mol natural cholesterol.

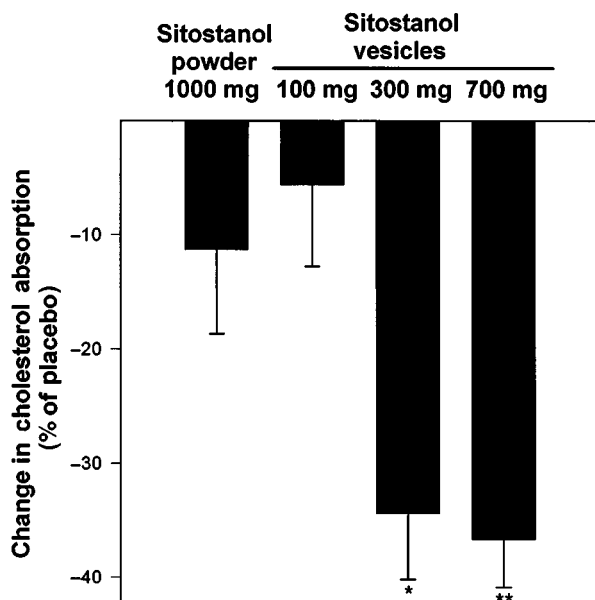


FIGURE 3. Mean (\pm SEM) reduction in cholesterol absorption due to sitostanol summarized from 3 clinical studies; $n = 5$ or 6 . Statistical significance was calculated independently for each of the studies as described in the Methods. **Significantly different from placebo: * $P = 0.01$, ** $P = 0.003$.

in LDL cholesterol when men consuming a low-cholesterol diet were dosed for 3 mo with 3.0 g sitostanol/d (11). In that study, sitostanol was suspended in oil at a final concentration of 20% by weight, whereas the solubility of sitostanol in oil is only 1% (24). Thus, most of the sitostanol was undissolved and may have had a microcrystalline structure resembling our powder. The ineffectiveness of sitostanol powder was predicted from our *in vitro* studies, which showed that sterols generally and sitostanol specifically were slow to dissolve in artificial bile (Table 2).

The solubility of sitostanol in artificial bile was greatly increased by including lecithin, suggesting a novel method for human administration that is consistent with current theory on the partitioning of sterols in the gut. Shown in **Figure 4** is an intestinal oil phase in equilibrium with an intestinal aqueous micellar phase, with cholesterol absorption occurring from the latter (7). Sitostanol or sitostanol ester powders equilibrate with the intestinal phases so slowly that they are ineffective except in very large amounts. We sonicated sitostanol with lecithin to obtain micelles that passed through a 5- μ m filter, enabling direct delivery into the intestinal micellar phase. This is an effective procedure because the micellar sitostanol preparation reduced cholesterol absorption by up to 37%. There was no significant difference between cholesterol reduction by 700 and 300 mg sitostanol, however, suggesting that a 37% reduction in cholesterol absorption was the most that could be achieved. A limit to the amount cholesterol absorption can be reduced was noted previously in animal studies (25) and the results achieved here are comparable with human experiments in which long-term administration of 3 g sitosterol/d in an aqueous suspension reduced cholesterol absorption by 47% (calculated from reference 9).

Our finding that the form in which phytosterol was presented in the small intestine determined its efficacy may help explain the

results of other clinical studies. Chronic feeding of 700 mg unesterified sitostanol/d in rapeseed oil had no significant effect on cholesterol absorption (26). This is consistent with the low solubility of sitostanol in oil and with substantial activity of sitostanol occurring in the micellar phase rather than the oil phase (Figure 4). In contrast, when 800 mg of the sitostanol moiety was administered daily as sitostanol ester in rapeseed oil, cholesterol absorption was reduced by 16.8% of the control value in one study and by 18.5% of the initial value in another (27). The greater solubility of sitostanol ester in oil and the effect of cholesterol esterase to cleave the ester and drive sitostanol into the micellar phase might have resulted in increased effectiveness.

The positive results with sitostanol esters have led to their commercial preparation in margarine. However, this delivery strategy requires consumption of 23–50 g dietary triacylglycerol/d (22), a large energy burden. On the other hand, presentation of sitostanol in lecithin micelles requires only a small amount of phospholipid and the micelles so formed are compatible with nonfat foods. The use of lecithin to solubilize sitostanol might re-create a more natural situation in which phytosterols are associated with phospholipids in plant cell membranes. In foods, cholesterol appears to be more closely associated with phospholipid than with triacylglycerol (28, 29); we speculate that therapeutic formulations containing lecithin might be more effective than those containing triacylglycerol in solubilizing phytosterols.

Our results suggest that the importance of natural dietary phytosterols in regulating cholesterol absorption needs to be reevaluated. The usual dietary phytosterol intake of 100–500 mg/d (30) is small compared with phytosterol doses (3–18 g/d) previously reported to be effective in reducing cholesterol absorption. As a result of this discrepancy, natural dietary phytosterols were thought to play a minor role in regulating cholesterol absorption. However, the evidence reported here shows that naturally occurring phytosterols should not be disregarded. An important study showed a strong inverse correlation between natural dietary phytosterol intake (\bar{x} : 279 mg/d) and percentage cholesterol absorption in middle-aged men (31). Taken together with our data, this suggests that naturally occurring phytosterols may significantly

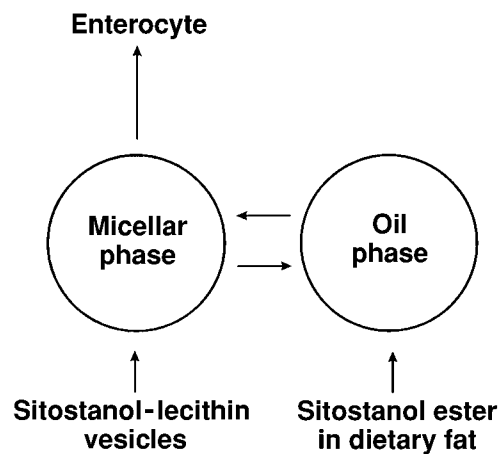



FIGURE 4. Interaction of sitostanol with intestinal phases. In this study, sitostanol was introduced directly into the intestinal micellar phase as a sitostanol-lecithin complex. Previous investigators fed sitostanol ester dissolved in dietary fat for entry into the oil phase (22). Unesterified sitostanol does not readily enter either the oil or the micellar phase.

affect cholesterol absorption, especially if presented with phospholipids. Because vegetable oils are the most concentrated source of dietary phytosterols (30), it is also possible that phytosterols account in part for the curious and unexplained ability of vegetable oils to lower LDL-cholesterol concentrations (32). 

We thank Norma Janes for dietary planning, Laura Kobayashi for study coordination, and Stephen Block for mass spectrometric measurements.

REFERENCES

- Pollak OJ. Reduction of blood cholesterol in man. *Circulation* 1953;7:702-6.
- Best MM, Duncan CH, Van Loon EJ, Wathen JD. The effects of sitosterol on serum lipids. *Am J Med* 1955;19:61-70.
- Heinemann T, Axtmann G, von Bergmann K. Comparison of intestinal absorption of cholesterol with different plant sterols in man. *Eur J Clin Invest* 1993;23:827-31.
- Sugano M, Morioka H, Ikeda I. A comparison of hypocholesterolemic activity of beta-sitosterol and beta-sitostanol in rats. *J Nutr* 1977;107:2011-9.
- Kesaniemi YA, Grundy SM. Turnover of low density lipoproteins during inhibition of cholesterol absorption by neomycin. *Arteriosclerosis* 1984;4:41-8.
- Wilson MD, Rudel LL. Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. *J Lipid Res* 1994;35:943-55.
- Grundy SM. Absorption and metabolism of dietary cholesterol. *Annu Rev Nutr* 1983;3:71-96.
- Hassan AS, Rampone AJ. Intestinal absorption and lymphatic transport of cholesterol and beta-sitostanol in the rat. *J Lipid Res* 1979;20:646-53.
- Lees AM, Mok HYI, Lees RS, McCluskey MA, Grundy SM. Plant sterols as cholesterol-lowering agents: clinical trials in patients with hypercholesterolemia and studies of sterol balance. *Atherosclerosis* 1977;28:325-38.
- Ling WH, Jones PJH. Dietary phytosterols: a review of metabolism, benefits and side effects. *Life Sci* 1995;57:195-206.
- Denke MA. Lack of efficacy of low-dose sitostanol therapy as an adjunct to a cholesterol-lowering diet in men with moderate hypercholesterolemia. *Am J Clin Nutr* 1995;61:392-6.
- Bosner MS, Ostlund RE Jr, Osofisan O, Grosklos J, Fritschle C, Lange LG. Assessment of percent cholesterol absorption in humans with stable isotopes. *J Lipid Res* 1993;34:1047-53.
- Ostlund RE Jr. A minimal model for human whole body cholesterol metabolism. *Am J Physiol* 1993;265:513-20.
- Goodman DS, Smith FR, Seplowitz AH, Ramakrishnan R, Dell RB. Prediction of the parameters of whole body cholesterol metabolism in humans. *J Lipid Res* 1980;21:699-713.
- Bosner MS, Lange LG, Stenson WF, Ostlund RE Jr. Percent cholesterol absorption in normal men and women quantified with dual stable isotopic tracers and negative ion mass spectrometry. *J Lipid Res* 1999;40:302-8.
- Ostlund RE Jr, Hsu F-F, Bosner MS, Stenson WF, Hachey DL. Quantification of cholesterol tracers by gas chromatography/negative ion chemical ionization mass spectrometry. *J Mass Spectrom* 1996;31:1291-6.
- Goodman DS, Noble RP. Turnover of plasma cholesterol in man. *J Clin Invest* 1968;47:231-41.
- Small DM. The physical chemistry of lipids. New York: Plenum Press, 1986:159-65.
- Armstrong MJ, Carey MC. Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. *J Lipid Res* 1987;28:1144-55.
- Grundy SM, Mok HYI. Determination of cholesterol absorption in man by intestinal perfusion. *J Lipid Res* 1977;18:263-71.
- Mattson FH, Grundy SM, Crouse JR. Optimizing the effect of plant sterols on cholesterol absorption in man. *Am J Clin Nutr* 1982;35:697-700.
- Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *N Engl J Med* 1995;333:1308-12.
- Crouse JR, Grundy SM. Evaluation of a continuous isotope feeding method for measurement of cholesterol absorption in man. *J Lipid Res* 1978;19:967-71.
- Vanhanen HT, Blomqvist S, Ehnholm C, et al. Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. *J Lipid Res* 1993;34:1535-44.
- Mattson FH, Volpenhein RA, Erickson BA. Effect of plant sterol esters on the absorption of dietary cholesterol. *J Nutr* 1977;107:1139-46.
- Miettinen TA, Vanhanen H. Dietary sitostanol related to absorption, synthesis and serum level of cholesterol in different apolipoprotein E phenotypes. *Atherosclerosis* 1994;105:217-26.
- Vanhanen HT, Kajander J, Lehtovirta H, Miettinen TA. Serum levels, absorption efficiency, faecal elimination and synthesis of cholesterol during increasing doses of dietary sitostanol esters in hypercholesterolaemic subjects. *Clin Sci* 1994;87:61-7.
- Hoelscher LM, Savell JW, Smith SB, Cross HR. Subcellular distribution of cholesterol within muscle and adipose tissues of beef loin steaks. *J Food Sci* 1988;53:718-22.
- Small DM, Oliva C, Tercyak A. Chemistry in the kitchen. Making ground meat more healthful. *N Engl J Med* 1991;324:73-7.
- Weirauch JL, Gardner JM. Sterol content of foods of plant origin. *J Am Diet Assoc* 1978;3:39-47.
- Miettinen TA, Kesaniemi YA. Cholesterol absorption: regulation of cholesterol synthesis and elimination and within-population variations of serum cholesterol levels. *Am J Clin Nutr* 1989;49:629-35.
- Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *N Engl J Med* 1986;314:745-8.