Nonesterified Phytosterols Dissolved and Recrystallized in Oil Reduce Plasma Cholesterol in Gerbils and Humans

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ABSTRACT When free phytosterols are adequately heated and then cooled in fat, they recrystallize and are rendered bioavailable for blocking cholesterol absorption. To extend the application of phytosterols to fried foods, the activity of these modified crystals was assessed in 2 experiments with 26 male gerbils fed purified diets containing 0.15 g/100 g cholesterol with or without 0.75 g/100 g free phytosterols. The heat-modified soybean sterols were added directly to the diet (Expt. 1) or as phytosterol-enriched potato chips (Expt. 2). In the gerbil experiments, only the diet containing phytosterols significantly reduced plasma cholesterol (35–48%) and the total cholesterol/HDL cholesterol (HDL-C) ratio (40%), as well as hepatic cholesterol esters (80%). In a subsequent human study, subjects ($n = 7$) consumed two 28-g servings of tortilla chips fried in oil with or without phytosterols that provided 0 or 1.5 g/d for 4-wk periods in a crossover design (Expt. 3). During consumption of the phytosterol-enriched chips, significant reductions in plasma cholesterol (10%) and LDL cholesterol (15%) were achieved without affecting HDL-C. This novel means of delivering free phytosterols proved to be both functionally efficient and effective. J. Nutr. 134: 1395–1399, 2004.

KEY WORDS: • *free phytosterols* • *plasma cholesterol* • *hepatic cholesterol* • *gerbils* • *humans*

The use of phytosterols to enrich a variety of foods has become an accepted means of lowering LDL cholesterol (LDL-C)[2] and total cholesterol (TC) since their initial successful use in margarines (1–6). Furthermore, it is noteworthy that LDL-C selectively declines, whereas HDL cholesterol (HDL-C) remains relatively constant with the addition of phytosterols to foods (6,7). On this basis, the National Cholesterol Education Program and American Heart Association recommended the addition of phytosterols to the daily diet of adults to help reduce plasma cholesterol (8); the FDA stated that foods containing sufficient plant stanols and sterols esterified with fatty acids can carry a heart healthy claim (9). On the other hand, because of limited solubility and questionable bioavailability, naturally occurring free sterols and stanols have been less studied and less well documented in terms of efficacy. Nevertheless, they appear to have the functionality of the esterified forms when appropriately presented in certain food fats (3,10).

A means of delivering phytosterols in foods other than margarines and salad oils would be attractive, providing they remain able to reduce LDL-C. Our objective with the present experiments was to define a means of adding free phytosterols to frying oil to extend the range of their beneficial effect to a variety of fried food products. Others have described various dispersal methods; the most common is to render the phytosterols fat-soluble by fatty acid esterification. Both free sterols and stanols derived from plant sources have been esterified and used successfully in margarines (1,2,4,6,11)

Several researchers tried with equivocal results to employ phytosterols in nonfat food systems, such as juices, drinks, or an occasional low-fat food (7,12–15). These experiments met with limited success presumably because of solubility problems, i.e., for the phytosterols to compete with cholesterol in the lipid micellar structure during fat absorption, they require an adequate degree of intestinal dispersal (4). Unlike cholesterol, however, only a small portion of the ingested phytosterols is absorbed. Biologically active phytosterols are thought to displace cholesterol in the micelle, leading to cholesterol excretion in the feces (16).

In the present studies, we initially explored a novel delivery system for free phytosterols in an animal model. This system was subsequently extended to humans in a clinical study with moderately hypercholesterolemic individuals who consumed corn chips fried in phytosterol-enriched oil and successfully lowered their LDL cholesterol.

MATERIALS AND METHODS

Animals, diets, and study designs

Experiment 1. The aim of the first study was to determine the hypocholesterolemic efficacy of free, i.e., nonesterified, prilled phytosterols (from soybean oil, ACH Companies) in an animal model responsive to dietary cholesterol. Male, 5-wk-old Mongolian gerbils ($n = 12$; Charles River) were housed in groups of 2–3 and kept in a controlled environment with a 12-h light:dark cycle (light on 1800 h). Gerbils were randomly assigned to 2 groups ($n = 6$) and fed for 4 wk purified diets containing 0.15 g/100 g cholesterol, with 30% energy provided by fat. The 2 diets contained either 0 or 0.75 g/100

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[2] Abbreviations used: apo, apolipoprotein; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; OSI, Oxidative Stability Index; TC, total cholesterol; TG, triglycerides; TRP, triglyceride-recrystallized-phytosterols.

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g phytosterols and 13.7 g/100 g fat (**Table 1**), meaning that the dietary fat of the test group contained 5.5 g/100 g of heat-dissolved phytosterols (0.75/13.7) such that the diet provided a 5:1 ratio of phytosterols:cholesterol. Composition of the soybean phytosterols was ~4% brassicasterol, 30% campesterol, 20% sigmasterol, and 40% β-sitosterol.

Because the solubility limit of free sterols in vegetable oil is <1.5%, the nonesterified phytosterols in all experiments were heated in the dietary fat to ensure their complete dissolution before formation of a phytosterol-complex. Specifically, when free phytosterols are dissolved in oil or fat (2–25 g/100 g) during heating up to 190°C, they subsequently recrystallize upon cooling to form a mixed crystalline composition that we have referred to as the "triglyceride-recrystallized-phytosterols" or (TRP)-complex (17). In this complex, the shape and size of the solid crystals are altered, and at 400X magnification, large plates and extended arrays of needle-like sterol crystals associated with fat (selectively stained with Sudan Black) are visible microscopically. These mixed crystalline phytosterol-triglyceride solids differ in their physical properties (lower remelting temperature and modified crystalline appearance) from finely milled, and/or microcrystalline particles that have not first been fully dissolved and intimately combined with a triglyceride-based fat or oil by heating and cooling. The degree of TRP-complex formation depends on both the concentration of phytosterols in the oil and the temperature achieved during heating.

For Expt. 1, this TRP-complex was mixed directly into the other dietary components as it cooled. All gerbils had free access to water, and food was provided daily in predetermined amounts to meet energy requirements for growth and maintenance. After 4 wk of feeding the experimental diets, gerbils were deprived of food overnight (16 h) and blood samples were collected into an EDTA-wetted syringe by cardiac puncture under light anesthesia. After exsanguination, the liver, right perirenal adipose, and cecum were excised and weighed. Plasma was separated from EDTA-treated blood by centrifugation at 12,000 × *g* for 15 min and analyzed within 1–2 d.

TABLE 1

Composition of the purified diets for gerbils (Expt. 1)[1]

Ingredient	Diet	
	Control (0% phytosterols)	Nonesterified phytosterols (0.75% prilled soybean)
	g/kg	
Casein	200	200
Sucrose	200	200
Cornstarch	296	289
Cellulose	100	100
Fat		
Coconut oil	81	81
Canola oil	43	43
Soybean oil	13	13
Mineral mix (Ausman-Hayes)[2]	50	50
Vitamin mix (Hayes-Cathcart)[3]	12	12
Choline chloride	3	3
Free phytosterols (prilled soybean)	0	7.5
Cholesterol	1.5	1.5

[1] Diets were fed as gel blocks, prepared by withholding from formulation 60 g/kg of cornstarch and premixing it with 800 mL of simmering water to form a gel when added to the remaining ingredients.

[2] Ausman-Hayes mineral mix F 8530, BioServe, g/kg of mix: potassium phosphate dibasic, 314; calcium carbonate, 290; sodium chloride, 162; magnesium sulfate, 99; calcium phosphate dibasic, 73; magnesium oxide, 32; ferric citrate, 27; manganese sulfate, 1.22; zinc chloride, 0.92; cupric sulfate, 0.29; potassium iodide, 0.077; chromium acetate, 0.044; sodium fluoride, 0.02; and sodium selenite, 0.004.

[3] Hayes-Cathcart vitamin mix, g/kg of mix: *dl*-α-tocopheryl acetate (500 IU/g), 15; inositol, 5; niacin, 3; calcium pantothenate, 1.6; retinyl palmitate (500,000 IU/g), 1.5; riboflavin, 0.700; thiamin, 0.600; pyridoxine HCL, 0.700; folic acid, 0.200; cholecalciferol (400,000 IU/g), 0.100; biotin, 0.020; cyanocobalamin, 0.001, menadione, 0.0002; and dextrin, 972.

Experiment 2. The aim of the second study was to evaluate the hypocholesterolemic efficacy of nonesterified soybean phytosterols fully dissolved directly in frying oil to prepare potato chips (i.e., to test the efficacy of the TRP-complex incorporated directly into chips). Most (4/5.5 = 73%) of the free phytosterols that were dissolved by superheating in oil recrystallized as the TRP-complex in chips that were then incorporated into the diet. The gerbil model developed in Expt. 1 was utilized for Expt. 2. Male, 5-wk-old gerbils (n = 14) were randomly assigned to 2 groups (n = 7) and fed for 4 wk purified diets containing 0.15 g/100 g cholesterol. Both diets contained 13.7 g/100 g fat (7.5 g from potato chips fried in canola oil plus 6.2 g as coconut oil), again with 30% dietary energy provided as fat. One potato chip batch contained free (nonesterified) sterols as 10 g/100 g of the fat; the other had none. The chips contained 24.1 g fat/100 g chips by analysis. These data were used to add 268 g of chips to 1 kg of diet, providing 0.75 g/100 g of phytosterols in the final diet. The control diet provided no sterols, but contained potato chips fried in regular canola oil. Final composition of diets was similar to Expt. 1, except that canola oil was more abundant and no soybean oil was added. Also, part of the fat (55% of total fat) and carbohydrate (64% of total), as well as all of the phytosterols, were added to the diets in potato chips. After final adjustments in oils, only slight differences were present between the polyunsaturated/saturated fat ratios of 0.27 (Expt. 1) and 0.38 (Expt. 2). All other experimental conditions, including animal maintenance, feeding, sample collection, and general methods approximated Expt. 1, except a portion of each liver was stored at −20°C until analyzed for cholesterol, and blood cholesterol analysis included subfractions.

Experiment 3. A clinical study was conducted to assess the feasibility of extending to humans the methods and technical information obtained from Expts. 1 and 2 with gerbils. To that end, a commercial manufacturer (Warnock Foods) prepared tortilla chips fried at 185°C in either high-oleic safflower oil or the same oil containing 12 g/100 g of phytosterols isolated from soybean oil (Archer Daniels Midland). In this process, it was discovered that the oxidative stability index, i.e., OSI (representing the time until fat breakdown during prolonged heating), was boosted almost 40% to a value of 15 h from an initial value of 11 h for the high-oleic safflower oil used for frying the tortilla chips. More importantly, after the commercial preparation of tortilla chips, the OSI of the residual phytosterol-fortified high-oleic safflower oil in the frying vessel had not decreased, whereas the OSI of the nonfortified oil had decreased almost 20%, from 11.3 h initially to 9.5 h during frying. Once processed, each bag of chips contained 750 mg of phytosterol as the TRP-complex, so that 2 bags of test chips provided 1.5 g phytosterol/d.

Subjects, primarily individuals with moderately elevated cholesterol (mean 6.0 mmol/L and range of 4.7 to 7.0 mmol/L) and not currently taking any medication or having any known clinical disease, were recruited from the campus community. A screening blood sample was used to identify persons with elevated cholesterol. This was followed by a baseline sample collected at d 0, if they qualified with the first blood sample. The mean of the 2 samples represented the baseline value for comparisons. Additional blood samples were collected after they consumed the chip diets for 3 and 4 wk to determine the final mean treatment effect, as measured by total cholesterol (TC), LDL-C, triglycerides (TG), and HDL-C (i.e., mean of wk 3 and 4 values) as described below. Vegetarians were excluded, and subjects were not asked to change their diet or exercise patterns, only to consume the two 28-g bags of tortilla chips as close to lunch and dinner as possible, preferably as part of the meal. Daily calendars were kept to record the consumption of chips and to serve as an index of compliance. In addition, subjects returned every 2 wk to receive their next allocation of chips and to discuss how the study was progressing for them with the primary investigator (K.C.H.). The study was designed to engage the 12 subjects eventually enrolled (8 men, 4 women with mean age of 48 ± 18 y) in a crossover compar-

ison of the 2 chips, with a washout period of at least 2 wk between treatments. Subjects were assigned randomly to the 2 groups, which were evaluated concurrently.

Of the 12 subjects initially assigned to either the control chips (no sterols) or test chips (with sterols), only 7 completed the crossover for various reasons. Two of the dropouts started with control chips, 3 with test chips. One man departed when he began medication for high cholesterol; a male student graduated before completing the second phase; one woman cited family problems and withdrew; one man left university employment and found it too inconvenient to continue; and the diary of one man suggested poor compliance during the last 2 wk, which was acknowledged with the explanation that he felt he was consuming too many extra calories. Otherwise, compliance was very good (>95%) and was supported by diary records and discussion of progress throughout the study with the primary investigator (K.C.H.). Both animal studies were approved by the IACUC, and the human study was approved by the IRB at Brandeis University.

Plasma lipid analysis. Plasma TC, HDL-C, and TG were measured by enzymatic assays (Sigma Diagnostics kits; procedures #352 for TC and #336 for TG). HDL-C in gerbils was assayed in the supernatant after the precipitation of lipoproteins containing apolipoproteins (apo) B and E with sodium phosphotungstate-Mg^{2+} (Boehringer Mannheim Diagnostics; procedure 543004), according to the procedure of Weingand and Daggy (18); for humans, HDL-C was assayed using Sigma procedure #352–4; and apo B-rich lipoproteins (LDL-C) were determined using the equation of Friedewald et al. (19).

Hepatic cholesterol analysis. Both free and esterified liver cholesterol were separated by HPLC according to the method of Kim and Chung (20) using a Waters Radial-Pack, C18 column eluted isocratically with acetonitrile:isopropanol (50:50, v:v) at 2.0 mL/min. Absorbance of the eluate was measured at 210 nm using a UV detector. Cholesterol concentrations (free and esterified) were calculated by comparing the peak areas for the samples with those obtained for the calibration standards (Sigma Chemical). To calculate esterified cholesterol, the sum of cholesteryl esters was divided by 1.67 (calculation according to Witztum et al. (21).

Statistical analysis. The Super ANOVA statistical package (Abacus Concepts) was used for all data comparisons. A two-tailed *t* test was used for the gerbil experiments. Because only 7 of 12 human subjects agreed to cross over to the opposite chips after completing their first 4-wk rotation, the data were analyzed in 2 ways to capture results from all 12 participants. First, their individual baseline lipid values were compared with values obtained after either 4 wk of chips plus sterols ($n = 10$) or after 4 wk of the sterol-free chips ($n = 9$), using a two-tailed *t* test. A second, statistically stronger, direct comparison represented a paired *t* test on the crossover data ($n = 7$), i.e., one in which each subject served as his/her own control for the 2 different chip rotations. Differences were considered significant at $P < 0.05$.

RESULTS

Experiment 1. Body weights did not differ between the 2 groups of gerbils, whereas plasma cholesterol was 35% lower ($P < 0.05$) and livers were significantly smaller (10%) in those receiving phytosterols. Cecal and adipose tissue weights and plasma TG were unaffected by the diets (**Table 2**).

Experiment 2. After 4 wk, body weights did not differ between the groups, whereas relative liver weight, liver cholesterol, and plasma lipid concentrations were all significantly reduced by phytosterols (**Table 3**). Specifically, the phytosterol-enriched potato chips reduced liver esterified cholesterol 76% and TC 48% compared with control chips without sterols. Plasma TG and HDL were not affected by diet, whereas the TC:HDL-C ratio was reduced 40% ($P < 0.05$) by phytosterols.

Experiment 3. Comparison of the 7 subjects who successfully completed both arms of the crossover (**Table 4**) revealed that TC and LDL-C, as well as the LDL-C:HDL-C ratio, declined significantly (10–15%) without lowering beneficial HDL-C, when the sterol-fortified chips were consumed. With each of these 7 crossover subjects, LDL-C decreased between 0.26 and 1.02 mmol. Because the number of dropouts was considerable, we sought support for the crossover results by applying a less stringent parallel design for all 12 subjects who completed at least 1 treatment period. Basal values for each individual were compared with his/her own end-of-treatment values. On that basis, no significant lipoprotein changes were observed between baseline and 4 wk when 9 subjects consumed control chips (e.g., LDL-C decreased 2%). This observation contrasted with the 10 subjects who consumed phytosterol-enriched chips for 4 wk and whose LDL-C decreased 15% from their entry value (data not shown). The latter decline was significant and comparable to the response for the subgroup of 7 subjects who crossed over between sterol-free chips and sterol-fortified chips. As intended with the study design, no diary or verbal inquiry revealed any change in dietary habit, physical activity, or body weight during the trial.

TABLE 2

Body and organ weights in gerbils that consumed diets containing 0 or 0.75 g/100 g nonesterified phytosterol for 4 wk (Expt. 1)[1]

	Diet group	
	Control (0% phytosterols)	Nonesterified phytosterols (0.75% prilled soybean)
Body weight, *g*		
Initial	53 ± 3	52 ± 2
Final	66 ± 4	65 ± 3
Relative organ weights, *g/100 g body*		
Liver	3.3 ± 0.2	2.8 ± 0.2*
Cecum	2.6 ± 0.6	2.5 ± 0.6
Adipose (perirenal)	0.32 ± 0.18	0.32 ± 0.10
Plasma, *mmol/L*		
TC	3.96 ± 0.18	2.56 ± 0.23*
TG	0.37 ± 0.11	0.27 ± 0.03

[1] Values are means ± SD, $n = 5–6$. * Different from the control group, $P < 0.05$.

DISCUSSION

These data indicate that free (nonesterified) phytosterols presented in a bioavailable form in the diet of mammals have a beneficial effect on plasma and liver lipids. Our results with soybean-derived free phytosterols in both gerbil experiments confirm earlier observations with tall oil–derived free phytosterols presented in the same TRP-complex (16). The cholesterol-lowering efficacy of phytosterols in fortified potato chips (Expt. 2) was similar to or even slightly better than that observed when phytosterols were provided in the dietary fat directly (i.e., fully dissolved by heating and then mixed with diet ingredients, Expt. 1), indicating that this application was feasible in a processed food. The clinical data further demonstrated that foods such as corn chips fried in a fat containing heat-solubilized, cooled, and recrystallized free phytosterols provide an effective means for reducing LDL-C in humans. Several others have indicated that free sterols prepared in various ways may or may not be effective (7,12–15,22). Our detailed description of heating and recrystallization in natural fat (17) delineates an exact procedure that appears critical to

TABLE 3

Body and organ weights and plasma and liver lipids of gerbils that consumed diets with potato chips containing 0.75 g/100 g nonesterified phytosterols for 4 wk (Expt. 2)[1]

	Diet group	
	Chips without phytosterols	Chips with nonesterified phytosterols (prilled soybean)
Body weight, *g*		
Initial	51 ± 4	51 ± 2
Final	66 ± 3	64 ± 2
Relative organ weights, *g/100 g body*		
Liver	3.1 ± 0.1	2.8 ± 0.1*
Cecum	2.7 ± 0.5	2.9 ± 0.4
Adipose (perirenal)	0.32 ± 0.11	0.38 ± 0.07
Liver cholesterol, *μmol/g*		
TC	101 ± 15	34 ± 10*
FC	13 ± 3	13 ± 1
EC	88 ± 16	21 ± 10*
Plasma lipids, *mmol/L*		
TC	4.91 ± 1.16	2.56 ± 0.28*
HDL-C	1.76 ± 0.23	1.50 ± 0.23
TG	0.58 ± 0.10	0.50 ± 0.07
TC:HDL-C	2.9 ± 1.1	1.7 ± 0.2*

[1] Values are means ± SD, $n = 5–7$, except liver cholesterol, $n = 4$.
* Different from the control group, $P < 0.05$.

optimizing free sterol function in vivo. Although the present application of phytosterols was demonstrated with fried chips, it could apply to other foods prepared with fat and phytosterols heated together to a specific temperature or by the addition of the preformed TRP-complex.

Nonesterified phytosterols have limited solubility (<1.5% by weight) in dietary fat, but when solid sterols were dissolved and recrystallized in fat heated to >70°C at a concentration of 10–12% by weight, the heating and cooling cycle allowed most of the plant sterols to form and be ingested as a uniquely composed TRP-complex (17). The degree to which the TRP-complex reduced liver and plasma cholesterol is noteworthy in light of the fact that free phytosterols (or even esterified sterols) either inadequately dispersed in fat (4,6,11,23) or simply added to foods, especially low-fat food or drink, (12,14,15,24) have marginal ability to lower plasma cholesterol. In gerbils, a dietary ratio of 5:1 between phytosterols and dietary cholesterol resulted in a 35–48% lower plasma cholesterol level with ~80% less liver cholesteryl ester accumulation, and a 40% reduction in the TC:HDL-C ratio. In our human subjects, the same phytosterol TRP-complex reduced LDL-C by 15%. Thus, the hypocholesterolemic efficacy of nonesterified plant sterols in the TRP-complex compares favorably with literature reports for humans consuming fat-soluble esterified sterols and stanols dispersed in fats, often at much higher intakes than our 1.5 g/d (1–6,25–27).

Generally one can expect an 8–15% decline in TC and LDL-C without affecting HDL-C when 1.7–3.0 g/d of phytosterol is successfully incorporated within a fat matrix, typically when esterified to fatty acids (1–6,10). Our degree of LDL-C reduction falls within the upper range of previously reported responses, indicating that the delivery vehicle was particularly effective. For humans, we provided a modest 1.5 g/d free phytosterols within fried chips (generally consumed with lunch and dinner) and obtained the rather maximal decline of 0.033 mmol LDL reduction/100 mg of phytosterol consumed. This is slightly better than the literature range of 0.022–0.031 mmol/100 mg of esterified phytosterols (4), and decidedly better than free phytosterols presented in fat-free (12), low-fat (14), or undispersed in oil (23) delivery systems.

Our favorable response presumably reflects the present form of delivery (TRP-complex) and possibly the timing of phytosterol intake. It has been argued for humans that dietary timing is less critical than the daily amount of phytosterols consumed (28). However, we observed previously in gerbils that efficacy for blocking cholesterol absorption was best when phytosterols and cholesterol were consumed together and that free phytosterols consumed in the absence of dietary fat or the TRP-complex failed to lower plasma or liver cholesterol effectively (16, unpublished data). In previous human studies related to these points (12,28), it is unclear whether the daily cholesterol intake coincided with phytosterol intake and whether the dietary mass ratio of phytosterols:cholesterol was considered in the study design.

Our previous gerbil study (16) revealed that the critical mass of heat-dissolved phytosterols required to achieve the greatest effect on TC and LDL-C was a 5:1 ratio of phytosterols to cholesterol. If more than a 5:1 dietary mass ratio was fed, no additional benefit was obtained in terms of liver and plasma cholesterol reduction. When a 3:1 dietary mass ratio was utilized, the phytosterols provided less effective protection against the dietary cholesterol challenge (unpublished data). Considering that the average human intake of cholesterol is ~300 mg/d, our provision of 1500 mg/d phytosterols in 2 bags of chips was calculated to provide maximum protection (assuming that the sterol micellar stoicheometry in humans is similar to gerbils, which the comparative data herein suggest is probable). The positive nature of these results indicates that

TABLE 4

Effect of tortilla chips, providing either 1.5 g/d or no phytosterols, on plasma lipids of humans after 4 wk (crossover trial; Expt. 3)[1]

Plasma, *mmol/L*	Baseline	Tortilla chips crossover		
		Without phytosterols	With phytosterols	% change[2]
TC	6.00 ± 0.93	5.90 ± 0.85	5.30 ± 0.88*	−10.2
HDL-C	1.24 ± 0.26	1.27 ± 0.25	1.27 ± 0.26	0
LDL-C	4.19 ± 1.06	4.06 ± 0.98	3.44 ± 1.06*	−14.7
TG	1.25 ± 0.56	1.24 ± 0.66	1.33 ± 0.52	+7.2
LDL-C:HDL-C	3.6 ± 1.3	3.4 ± 1.2	2.9 ± 1.2*	−10.2

[1] Values are mean ± SD, $n = 7$. * Different from group without phytosterols, $P < 0.05$.
[2] Change between chip periods.

our delivery method for phytosterols in a solid processed food is effective. Having a variety of solid food vehicles for phytosterol delivery should enhance dietary compliance compared with relying solely on a margarine or spread. In addition, free phytosterol supplementation in gerbils actually *reduced* the endogenous hepatic phytosterol content, suggesting that this form of supplement reduces the accumulation of both hepatic cholesterol and phytosterols (16).

A serendipitous observation from these studies, detailed in the Materials and Methods and elsewhere (17), is the observation that vegetable oils fortified with free phytosterols are substantially stabilized against oxidation during heating (and subsequent rancidity during storage). Because oxidized fats are thought to have a detrimental effect on lipoproteins, acting to promote atherogenesis once the oxidized products are absorbed (29,30), the secondary health benefits of stabilizing frying oils against oxidation using natural phytosterols could be substantial.

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