



May 18, 2004

Dockets Management Branch
Food and Drug Administration
5630 Fishers lane, Room 1061 (HFA-305)
Rockville, MD, 20852

Re: Cholesterol lowering efficacy of free, non-esterified phytosterols in low and fat-free foods

In response to a recent study that suggested that free, non-esterified phytosterols are ineffective in reducing blood cholesterol we have reviewed the entire body of literature on free phytosterols and blood cholesterol reduction. We conclude that non-esterified phytosterols are efficacious in low and fat-free foods and are equally effective as phytosterol esters in lowering blood cholesterol.

It has been well established that phytosterols lower total and LDL cholesterol. Studies of their efficacy date back to the 1950s when high doses were used as cholesterol-lowering drugs. Early phytosterol preparations were not highly available, requiring large doses to ensure adequate effect. In the late 1970s esterification of phytosterols with fatty acids was discovered as a process to make them more soluble in dietary fats, enabling the use of much lower phytosterol doses (i.e., 1-3 grams). Esterified phytosterols are readily incorporated into fat-based food matrices such as margarine, fat spreads, and salad dressings. Esterified plant sterols and stanols however, are not well suited for low fat or fat-free food formulations. In keeping with current heart healthy dietary recommendations, consumers should have the option to use low fat or fat-free phytosterol containing foods. Recent technological advances now permit low doses of free, non-esterified phytosterols to be incorporated into a wide range of low fat and fat-free products.

Several recent studies have confirmed that non-esterified phytosterols reduce blood cholesterol levels in people with normal to elevated cholesterol levels consuming usual diets or reduced fat diets. We acknowledge that one recent study, conducted by Jones et al (2003), reported that 1.8 g of unesterified phytosterols incorporated into low or non-fat beverages did not lower blood cholesterol levels compared to a non-fat control beverage. These researchers concluded that the cholesterol-lowering potential of phytosterols may depend on their previous dispersion into a fat matrix and on the physical nature of the food, although they provided few little information on their preparation process or beverage composition. As discussed below however, the study's experimental design raises concerns and would tend to invalidate Jones's conclusions based on the reported findings. Clearly, the results of the Jones study cannot be generalized to other low fat or fat-free foods and beverages.

Efficacy of Non-esterified Phytosterols in Low and Fat-Free Foods

Six recently published human studies have evaluated the effect of non-esterified phytosterols in low fat or fat-free beverages on serum lipids or cholesterol absorption. Five of these studies reported positive results with the addition of free phytosterols to products such as orange juice, low fat milk, lemonade, a low fat yogurt drink, and a Crystal Lite drink (Devaraj et al, 2004; Poteau et al, 2003; Spilburg et al, 2003; Volpe et al, 2001; Ostlund et al, 1999). Jones et al (2003) was the only study that did not observe a beneficial effect on serum lipids with phytosterol-supplemented fat-free and low fat beverages. With the exception of Ostlund et al (1999), these studies utilized a phytosterol dose in the 2 g range.

Subjects with mildly elevated cholesterol levels (n=72) participated in the placebo-controlled, double-blind, randomized trial conducted by Devaraj et al (2004). After a 2-week run-in phase, subjects consumed either orange juice placebo or orange juice to which 1 gram per 8 fluid ounces of free phytosterol was added was consumed twice a day with meals (OJ sterol group). Otherwise, all subjects continued their normal diets. A proprietary technique (pending patent) was used to suspend the phytosterols with a specific particle size in orange juice. Total cholesterol in the OJ sterol group was reduced by 7.2% and LDL cholesterol by 12.4% compared to baseline ($p < 0.01$) and was significantly reduced compared to the orange juice placebo group ($p < 0.01$). In addition, apolipoprotein B levels were also significantly decreased (9.5%) with the OJ sterol group ($p < 0.01$). These results clearly demonstrate the efficacy of free phytosterol in a fat-free food vehicle.

Poteau et al (2003) in a double blind, randomized, cross-over trial using staple isotopes demonstrated that 1.8 g of unesterified soy plant sterols solubilized in low fat milk, significantly lowered cholesterol absorption from 70 to 41% in 16 hypercholesterolemic subjects compared to a control low fat milk ($p < 0.001$). The phytosterol milk product also inhibited the uptake of cholesterol in intestinal Caco-2 cells *in vitro*. Plant sterols in the milk matrix were stabilized using a proprietary crystal retardation and emulsification system. A filtration assay found that only 1.3% of the added sterols could not pass through an 11 μ m filter in the presence of bile salts and lysophospholipids. These investigators predict that any phytosterol crystals that pass through an 11 μ m filter have a higher probability of becoming resolubilized in the intestine, since microcrystalline plant sterol preparations are known to lower LDL cholesterol *in vivo* (Christiansen et al, 2001).

The objective of the two-part study by Spilburg et al (2003) was to show that fat free foods supplemented with soy stanol-lecithin lower cholesterol absorption and serum LDL cholesterol in normal and mildly hypercholesterolemic subjects. Acute cholesterol absorption was measured in paired single-meal tests with or without soy stanols in 21 subjects. The test meals contained a lemonade beverage or egg whites that contained 625 mg free, non-esterified stanols emulsified with a small amount of lecithin (550 mg). Cholesterol absorption was reduced by 32.1% ($p=0.0045$, n=10) and by 38.2% ($p=0.0022$, n=11) by stanol-supplemented

lemonade and egg whites respectively. Consumption of the soy stanol lemonade beverage three times a day (1.9 g stanols/day) for 4 weeks in conjunction with the AHA Step 1 diet lowered total serum cholesterol by 10.1% ($p=0.0019$) and LDL cholesterol by 14.3% ($p=0.0016$) in 24 subjects compared to a placebo lemonade. Spilburg and coworkers state that their study demonstrates that

“free, unesterified stanols can be formulated with lecithin to produce a powder that can be readily dispersed in water, providing a flexible delivery system that is amenable to many different food types”.

In the double-blind crossover study by Volpe et al (2001), hypercholesterolemic subjects on a NCEP Step 1 diet consumed a low fat yogurt drink (control) or a low fat yogurt drink with 1 g/day of unesterified soy sterols for 4 weeks. After a 2-week washout period, subjects participated in another crossover study in which they consumed the control yogurt drink or a yogurt drink fortified with 2g/day of soybean sterols for 8 weeks. Plant sterols significantly reduced serum total cholesterol and LDL cholesterol in a dose dependent manner. During the first phase, total cholesterol and LDL cholesterol was reduced 7% ($P=0.0005$) and 11% ($P = 0.0009$) respectively, compared to baseline; in the second phase, total cholesterol and LDL cholesterol were lowered by 11% ($P < 0.001$) and 16% ($P < 0.001$) respectively, relative to baseline. Consumption of the placebo did not significantly lower serum cholesterol concentrations relative to baseline levels.

Ostlund et al (1999) first reported in a pilot study of 6 healthy subjects that sitostanol in lecithin micelles reduced cholesterol absorption by 36.7% ($p=0.003$) at a dose of 700 mg and 34.4% ($p=0.01$) at a dose of 300 mg. In contrast, 1g of sitostanol powder reduced cholesterol absorption by only 11.3%. The phytosterol preparation contained a 3:1 molar ratio of soy lecithin and sitostanol, which was diluted in water and flavored with Crystal Lite and consumed by subjects as a beverage. These researchers note that esterification of phytosterols increases solubility in fat products, but require the consumption of 23-50 grams of additional fat. Preparation of sitostanol in lecithin micelles requires only a small amount of phospholipids and the micelles formed are compatible with nonfat foods.

Jones et al (2003) fed 15 mildly hypercholesterolemic subjects a fat-free placebo beverage (NF), a fat-free beverage with free phytosterols (NFPS), or a low fat beverage with free phytosterols (LFPS) for 3 weeks in a random order crossover design. Total cholesterol (TC) concentrations were reduced 8.5%, 11.6% and 10.1% from baselines levels with NF, NFPS, and LFPS consumption, respectively. LDL cholesterol levels were reduced 5%, 10%, and 8.5% respectively by the same groups. Differences between groups were not statistically significant for total or LDL cholesterol, largely due to serum cholesterol reductions in the placebo group. However, total and LDL cholesterol were significantly lower compared to baseline in all groups ($p<0.05$). The significant changes in the placebo group suggest that subject's cholesterol levels were not stable with respect to the controlled diet. It is not clear that the model was capable of showing a difference between the placebo and phytosterol groups. Also the number of subjects was relatively small (15) and the length of intervention short (3 weeks). This study also showed a smaller LDL

cholesterol reduction than total cholesterol, the opposite of most phytosterol studies. The 1.8 g/day mixture of phytosterols used in this study was derived from tall oil. No other information was provided regarding its manufacture or its functional characteristics. In view of the effectiveness of free phytosterol in the test groups and the other circumstances, the authors generalized conclusion that “unesterified phytosterols, when provided in low fat and nonfat beverages do not lead to greater reductions in TC and LDL cholesterol compared with a control diet” is a substantial over generalization. Furthermore, their conclusion is contradicted by the finding of the other studies cited above.

Other studies also support the efficacy of unesterified phytosterols in low or fat-free non-beverage formulations (Tikkanen et al, 2001; Heinemann et al, 1986; Beer et al, unpublished).

Tikkanen et al (2001) randomly allocated 78 subjects with mild to moderate hypercholesterolemia to a placebo or treatment group providing bread, meat products, and jam in yogurt enriched with 1.25 to 5 g/day free plant sterols. (The nutrient composition of these foods was not reported, but the researchers indicated them to be low fat or fat-free foods). The wood phytosterol mixture was pulverized to a particle size < 130 µm to allow it to be evenly incorporated into the food items. Each of the three food types provided one third of the phytosterol dose. After 15 weeks, total cholesterol was reduced by 8% in the plant sterol group compared to 3% in the placebo group (p=0.0017) and LDL cholesterol was reduced by 13% compared to 5% in the placebo group (p=0.0070). These researchers noted that this study shows that unesterified plant sterols lower elevated serum cholesterol as effectively as plant sterol esters ingested as part of margarine. They state

“the cholesterol-lowering efficacy may not depend on esterification and solubility in food fats but rather on the thorough mixing of the plant sterols with intestinal contents, providing access to mixed micelles.”

As early as the mid-1980s, Heinemann et al (1986) demonstrated that a low dose of non-esterified sitostanol (1.5 g/day) consumed as capsules for 4 weeks was able to lower total cholesterol by 15% (p < 0.001) in hypercholesterolemic patients compared to a control period without phytosterols. Each stomach-soluble capsule contained 250 mg sitostanol partly dissolved and partly dispersed in 368 mg sunflower oil. This study included only 6 subjects and was not randomized, or placebo-controlled.

During the comment period for the interim final health claim rule for plant sterol/stanol esters, FDA received comments from manufacturers supporting the efficacy of unesterified phytosterols in several unpublished studies (some of which have since been published). The Altus Food Company (2001) reported a study that tested 1.8 g free tall oil sterols incorporated into low fat cereal bars consumed by 131 mildly hypercholesterolemic subjects for 8 weeks (Beer et al, unpublished a). Both total and LDL cholesterol was reduced by 6% in the phytosterol group compared to the placebo group. No significant effects were observed for HDL

cholesterol or triglycerides. This study utilized a double blind, randomized parallel study design that evaluated treatment effects within the context of a low fat diet.

Efficacy of Non-esterified Phytosterols Compared to Phytosterol Esters

Numerous studies have shown that non-esterified phytosterols are as effective as phytosterol esters. The seven studies reported in the previous section, each of which utilized low fat or fat-free formulations, clearly demonstrate that esterification of phytosterols are not necessary to induce significant cholesterol lowering effects. Ten additional studies are supportive of the same conclusion in other food formulations (Gremaud et al, 2002; Graaf et al, 2002; Vanstone et al, 2002; Christiansen et al, 2002; Nestel et al, 2001; Meguro et al, 2001; Sierksma et al, 1999; Jones et al, 1999; Pelletier et al, 1995; Mattson et al, 1982). In addition to the study by Jones et al (2003), only two other studies (Mittinen and Vanhanen, 1994, and Denke et al, 1995) did not observe a beneficial effect of non-esterified phytosterols.

Gremaud et al (2002) observed a beverage containing approximately 3 g/d of non-esterified stanols solubilized in lecithin and vegetable oil and emulsified in water consumed for 3 days by hypercholesterolemic subjects lowered cholesterol absorption by 55.7% during the test phase compared to 33.5% during the control phase of a randomized, double blind, crossover study ($p < 0.01$). The fat content of the phytosterol supplemented beverage was not reported and therefore it is not known whether it was a low fat product.

De Graaf et al (2002) utilizing a randomized, double-blind, placebo-controlled trial reported total and LDL cholesterol were significantly reduced by 6.4% ($p < 0.001$) and 10.3% ($p < 0.001$) in hypercholesterolemics who consumed 1.8 g unesterified phytosterols in a chocolate matrix for 4 weeks.

Vanstone et al (2002) reported four dietary treatments were fed to hypercholesterolemic subjects in a randomized crossover study for 3 weeks: 1.8 g/day non-esterified plant sterols (NS), 1.8 g/day non-esterified plant stanols (SS), 1.8 g/day of non-esterified plant sterols and stanols in a 50:50 mixture (NSS), cornstarch control. Non-esterified plant sterols and stanols were blended into butter. At the end of the supplementation phase, total cholesterol were 7.8%, 11.9%, and 13.1% lower ($p < 0.01$) in the NS, SS, and NSS groups respectively compared to the control group. LDL cholesterol values were 11.3%, 13.4%, and 16.0% lower ($p < 0.03$) in the NS, SS, and NSS groups respectively.

In a 6-month double-blind, randomized, placebo-controlled study, Christiansen et al (2001) fed hypercholesterolemic subjects 1.5g/day or 3 g/day of microcrystalline plant sterols in a rapeseed oil spread. Total cholesterol levels were 8.9% and 8.3% lower than those of the control group ($p=0.001$), respectively. LDL cholesterol was reduced by 11.3% and 10.6% by the 1.5 g and 3 g doses ($p=0.002$). The investigators note that

“plant sterols in microcrystalline form are as effective as in a fat soluble ester form. Due to the microcrystalline structure of this ingredient, the effective surface areas of the plant sterol crystals is large and thus achieves a highly effective trapping of cholesterol molecules in the intestinal lumen. Because plant sterols are in the free form, no hydrolyzing is required before this effect can be achieved.”

Meguro et al (2001) found a dose of 500 mg/day of free non-esterified sterols dissolved in diacylglycerol and formulated in mayonnaise significantly reduced LDL cholesterol in moderately hypercholesterolemic subjects who consumed the test product for 2 weeks ($p < 0.05$). Non-esterified sterols dispersed in triacylglycerol did not lower LDL cholesterol because the researchers report that the sterols were not well solubilized in triacylglycerol.

LDL cholesterol was reduced by 24.4% in the phytosterol group compared to 8.9% ($p < 0.001$) in the control group in the placebo-controlled, parallel study by Jones et al (1999). These results were observed after hyperlipidemic men consumed 1.7 g of a non-esterified phytosterol mixture of 20% stanols and 80% sterols for 30 days in a margarine spread.

Sierksma et al (1999) reported significant reductions of plasma total and LDL cholesterol of 3.8% and 6% respectively in a double-blind crossover study of healthy adult volunteers after they had consumed 0.8 g/day of free soybean sterols in soybean oil spread for 3 weeks ($p < 0.05$ for both). The investigators conclude their findings are similar to those of Hendriks et al (1999) who fed normo- and mildly hypercholesterolemic subjects a spread enriched with esterified soybean sterols at a similar intake of 0.8g/day and found total and LDL cholesterol were reduced by 4.9% and 6.7% respectively.

Healthy male subjects were fed 740 mg of non-esterified plant sterols in butter for 4 weeks in a randomized, placebo-controlled crossover study by Pelletier et al (1999). Total blood cholesterol was significantly lowered by 10% and LDL cholesterol by 15% compared to the control period ($p < 0.001$ for both).

Denke et al (1995) found 3 months of 3 g/day of sitostanol supplementation did not significantly lower LDL cholesterol in 33 men with moderate hypercholesterolemia who were consuming a low cholesterol diet. Sitostanol was suspended in safflower oil and packed into gelatin capsules, each containing 250 mg sitostanol and 1 g safflower oil. Some have suggested the lack of effect observed in this study may be due to sitostanol insolubility (Ostlund et al, 1999) or sitostanol powder crystals that were too large to have been adequately dispersed (Poteau et al, 2003).

Three clinical studies (Nestel et al, 2001; Miettinen and Vanhanen, 1994; and Mattson et al, 1982) and one animal study (Hayes, 2002) have made direct comparisons between non-esterified and esterified phytosterols. Nestel et al (2001) fed hypercholesterolemic subjects 2.4 g of plant sterols in esterified form or 2.4 g of non-esterified plant stanol for 4 weeks in a randomized, controlled

crossover study. At the end of the intervention period, median total cholesterol was reduced by 8.5% ($p < 0.001$) by the sterol esters and 3.5% ($P < 0.001$) by the free stanols. Changes in total cholesterol was significantly different between sterol esters and free stanols ($p = 0.05$), but median LDL cholesterol was lowered by 13.6% and 8.3% ($P < 0.003$ for both) by sterol esters and free stanols respectively, and were not statistically different. The researchers state the differentiation between esterified and non-esterified phytosterols may be minor.

Thirty one hypercholesterolemic male and female subjects were randomized into four groups and received specific phytosterols dissolved in mayonnaise: (1) control, no phytosterols; (2) 0.7 g/day of free sitosterol; (3) 0.7 g/day of free sitostanol; (4) 0.8 g/day of esterified sitostanol (Mittinen and Vanhanen, 1994). All groups consumed mayonnaise preparations for 9 weeks. Decreases in total and LDL cholesterol were significant only for the sitostanol ester group (3.4% and 5.9% respectively, $p < 0.05$). This study did not make an appropriate comparison between esterified and non-esterified sitostanol because the doses were not equivalent, and the dose used for the free phytosterols is lower than the minimal dose reported to be clinically efficacious (i.e. 0.74- 0.8 g/day).

Cholesterol absorption differences were evaluated by Mattson et al (1982) who fed nine subjects on three separate occasions, a high cholesterol meal with 1 g of free sitosterol, 2 g of beta-sitosteryl oleate (i.e. esterified), or a no additive control. Free sitosterol decreased cholesterol absorption by 42% and beta-sitosteryl oleate reduced absorption by 33%. While this study demonstrates that both free and esterified phytosterols can reduce cholesterol absorption, it does not clearly address the difference of non-esterification versus esterification because 2 g of the sterol ester (1.6 g free sterol) is not equivalent to 1 g of free sterol.

In a study of gerbils fed diets containing cholesterol, Hayes et al (2002) showed that free phytosterols dissolved in fat were equally effective in blocking cholesterol absorption and lowering plasma and liver cholesterol as similar amounts of phytosterols that were esterified and formulated in margarines. The researchers note that

“the free sterol is the active form that presumably displaces (out competes) free cholesterol in gut micelles; and although esters are typically more readily dispersed in fat, they must first undergo hydrolysis to be active in the gut. A critical consideration in this context is to ensure adequate dispersal of the active sterol in the diet and gut. Based on the presumed mechanism of competing for space in the micelle, the tradeoff between sterol forms reflects their improved fat solubility by esterification countered by their need for hydrolysis once in the gut vs. the relatively poor solubility of the active free sterols/stanols in dietary fat. Dispersal and incorporation of the free sterols/stanols into micelles apparently was efficient in our experiments because the free sterol preparation proved equivalent, if not superior to the esterified forms provided in the spreads.”

Indirect comparisons made by researchers and reviewers support the equivalent clinical efficacy of free non-esterified phytosterols with their esterified counterparts. Spilburg et al (2003) observed an LDL lowering of 14.3% relative to controls in their study 1.9 g of unesterified stanols emulsified with small amounts of lecithin. These investigators state their results

“compares favorably with values observed previously with stanol margarines. Using that delivery system, 1.6 g/day of stanols delivered as esters reduced LDL by 6.8% (Hallikainen et al, 2000), 1.8 g/day by 9.8% (Miettinen et al, 1995), 2.3 g/day by 10.3% (Hallikainen et al, 2000), 2.8 g/day by 15% (Miettinen et al, 1995), and 3.0 g/day by 11.3% (Hallikainen et al, 2000). Thus the effect observed here is in the upper range of reported values for all doses of stanols....Because we used only free stanols, it is also apparent that neither esterification nor prior solubilization in fat are required to produce a good effect.”

Plat et al (2000) in a review of therapeutic potential of plant sterols and stanols indicate...

“For plant sterols, esterification does not change its LDL cholesterol lowering effects. This can be concluded from the results of Hendriks et al (1999) who found an LDL cholesterol reduction of 6.2% after the consumption of 0.83 plant sterol esters, whereas in a later study (Sierksma et al, 1999) a reduction in LDL cholesterol of 6.0% was reported after the consumption of 0.8 g free plant sterols.”

Similarly, Moreau et al (2002) in a comprehensive review of phytosterols report:

“In light of... recent research of Jones et al (1999) and others (Vanstone et al, in press; Christiansen et al, 2001), it may be argued now that properly formulated free phytosterols and stanols may be as effective as stanyl and steryl esters for lowering LDL-C levels in humans. This seems to be confirmed in a recent study by Nestel et al (2001), who compared free stanols and phytosteryl esters head-to-head.....While more research remains to be done to completely resolve this question, a current analysis of recent research appears to indicate that free sterols and stanols and steryl and stanyl esters, formulated in a manner that makes them “equally bioavailable,” all may give similar effects on LDL-C levels.”

Conclusions

Overall, the data show a consistent, statistically and clinically significant cholesterol lowering effect of free, non-esterified plant stanols and sterols in a variety of formulations that include low fat and fat-free food matrices. The effect was observed as to both total and LDL cholesterol, in hypercholesterolemics and normocholesterolemics. Free unesterified phytosterols lowered blood cholesterol

levels in those consuming their usual diet, as well as those on a low fat diet. These findings are consistent with those observed with phytosterol esters.

Sincerely,

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Enclosures

cc: Barbara Schneeman, Ph.D.
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