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Research

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The lipid lowering effect of plant sterol ester capsules in hypercholesterolemic subjects

Robert V Acuff¹, David J Cai^{*2}, Zhi-Ping Dong¹ and Doris Bell³

Address: ¹East Tennessee State University, College of Medicine, Johnson City, TN, USA, ²Cognis Corporation, LaGrange, IL, USA and ³Cognis Deutschland GmbH & Co. KG, Monheim, Germany

Email: Robert V Acuff - acuffr@etsu.edu; David J Cai* - david.cai@cognis.com; Zhi-Ping Dong - dong@etsu.edu; Doris Bell - doris.bell@cognis.com

* Corresponding author

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Abstract

Background: Foods enriched with phytosterols have been proven to be an effective therapy to improve blood lipid profiles. However, none of the studies have investigated the efficacy in lipid lowering of plant sterol esters (PSE) in capsule form. The objective of this study is to determine if the plant sterol esters (PSE) in capsule form (1.3 grams of PSE/day) lowered plasma cholesterol levels and lipid ratios in free-living hypercholesterolemic subjects during a 4-week intervention period.

Methods: Sixteen subjects participated in a double-blind, placebo-controlled, sequential study with a 4-week placebo phase followed by a 2-week wash-out period and a 4-week treatment phase. Subjects were instructed to maintain stable diet pattern and physical activities. Blood samples were collected at 7, 21 and 28 days of each phase. The primary measurements were change in plasma total cholesterol (TC), HDL-cholesterol (HDL) and LDL-cholesterol (LDL) between phases and within each phase. The secondary measurements were change in triglycerides, lipoprotein ratios (TC/HDL, LDL/HDL) and C-reactive protein (CRP).

Results: In comparison to placebo, LDL-cholesterol was significantly reduced by 7% and 4% ($P < 0.05$) at both week 3 and week 4; HDL at week 3 of the treatment was significantly increased by 9% ($P < 0.01$), but not at week 4 (4%); total cholesterol was not significantly different from placebo throughout the period, TC/HDL and LDL/HDL were significantly reduced by (8%, 8%, 6%, 10%, respectively) ($P < 0.01$) at both week 3 and week 4. CRP and triglycerides did not differ either between the two phases or during the treatment phase.

Conclusion: In conclusion, plant sterol ester capsule is effective in improving lipid profiles among hypercholesterolemic subjects in a free-living setting at the minimum dosage recommended by FDA. The significant improved lipid profiles were reached after three weeks of administration. To achieve better lipid lowering results, higher dosages and combination with diets low in saturated fat and cholesterol are recommended.

Background

Elevated LDL-cholesterol is a significant risk factor for cor-

onary artery disease. The use of statin drugs is the current therapeutic option for lowering LDL-cholesterol and

improving lipid profile in hypercholesteremic patients. According to the American Heart Association guidelines, other options should be considered as well to treat or prevent hypercholesterolemia, including the use of phytosterols. Phytosterols, either as plant sterols or plant stanols, are natural cholesterol-like substances derived from plants [1]. The main mechanism by which phytosterols reduce blood cholesterol is to inhibit cholesterol absorption in the small intestine. Therefore, the physical forms, carriers and solubilization of the phytosterols are important characteristics to determine the efficacy of phytosterols on cholesterol lowering [2].

In terms of physical forms, free phytosterols are water and oil insoluble. The efficacy on lowering blood cholesterol of free phytosterols is often dependant on the dispersion capability in water and oil [3-5]. Recently, soy lecithin has been used to form more dispersible complexes with free sterol or stanol resulting in more bioavailable free sterol/stanol formulation than previous formulations [6-8]. However, the improved efficacy has only been confirmed in tablet forms, but not in the capsule form [7,8]. Fatty acid esters of sterols or stanols, on the other hand, are oil soluble. Thus, they are more easily dispersible in oils than free sterols or stanols which make them a better choice for soft gel capsules than the free sterol/stanol.

In terms of carriers, there is abundant evidence suggesting LDL-cholesterol lowering efficacy of phytosterols either as plant sterols or stanols in food forms, including water emulsions [3]; water as lecithin micelles [9]; yogurt [10,11]; low fat milk [12,13]; chocolate [14]; cereal; snack bars, breads, and beverages [15,16]. However, there are very few studies that investigated if these compounds provided as pharmaceutical forms, such as tablets and capsules, offer the same benefits [2,7,8]. The information on non-food forms is essential for long term supplementation strategy. As indicated by Law et al, (1994 and 2003), to achieve life saving benefits from mortality associated with heart disease and stroke, the LDL-cholesterol lowering strategy needs to be maintained for at least two years, and preferably for five years [17,18]. The pharmaceutical dosage forms, such as tablets and capsules, can provide more convenience and flexibility needed for the recommended long term usage than the traditional food applications [2]. Furthermore, these forms could be better delivery vehicles for phytosterols to be incorporated into the combination therapeutic strategy with pharmaceutical agents to provide an additional LDL-cholesterol lowering effect [19-21]. Unfortunately, none of the studies with these pharmaceutical forms used plant sterol esters [2,8]. Considering plant sterol ester is a more dispersible form in oil than free phytosterols, phytosterol esters may be more suitable choice for soft gel capsules than free plant sterols/stanols.

The present study was designed to confirm the efficacy of the plant sterol esters (PSE) in capsule form on lipid profile lowering in free-living hypercholesterolemic subjects during a short term (4 weeks). The dosage used in the study, 1.3 g plant sterol ester (0.8 g free sterol equivalent), is the minimum dosage that is recommended by FDA's health claim [22]. The information will be essential for usage of plant sterol esters in supplement forms beyond current food applications.

Results

No subject was dismissed because of the inability to tolerate the treatment or placebo or because of an adverse action or event. The statistical evaluation was determined based upon the 16 remaining subjects who completed the total study.

Placebo vs. treatment

Total cholesterol was reduced, but not significantly, at the end of treatment period (5%) ($P = 0.07$) (Table 1, Figure 1). LDL-cholesterol, TC/HDL, LDL/HDL ratios were significantly reduced by 7% ($P < 0.05$), 8% ($P < 0.01$) and 6% ($P < 0.01$) at day 21, 4% ($P < 0.05$), 8% ($P < 0.01$) and 10% ($P < 0.01$) at day 28, respectively (Table 1, Figure 1). HDL at day 21 of the treatment was significantly higher in comparison to placebo (9%, $P < 0.01$), but not at day 28 (4%) (Table 1, Figure 2). CRP and triglycerides did not differ between placebo and treatment phase.

Within each phase

There was no significant change in any of the measurements within the placebo phase (Table 1). Within the treatment phase, in comparison to day 7, LDL was significantly reduced at day 21 ($P < 0.05$), but not at day 28 (Table 1, Figure 1); HDL was significantly increased at both day 21 and 28 ($P < 0.05$) (Table 1, Figure 2); TC/HDL and LDL/HDL ratios were significantly reduced at both day 21 and 28 ($P < 0.001$) (Table 1). Total cholesterol was reduced throughout the treatment phase, but this reduction was not significant. CRP and triglycerides did not change during the treatment phase.

Discussion

The cholesterol lowering effect of plant sterols, either as free or esterified forms, is well documented in the literature. Most studies used food forms as delivery vehicles. There are only a few studies that have evaluated the effectiveness of phytosterols in tablet or capsule form, all of which used free stanols [7,8,23]. To our knowledge, the present study is the first study to demonstrate a significant reduction in plasma LDL cholesterol by 4% with plant sterol esters in capsules ($P < 0.05$). Epidemiological studies have shown that such a 4–5% reduction of LDL correlates with a 5–10% reduction in CHD risk in the first 5 years, and by 10% over a life time [24]. This result indi-

Table 1: Effects of plant sterol ester capsules on plasma lipid profiles and CRP (n = 16)¹

Parameters	Initial Baseline ²	Day 7	Day 21	Δ PSE-Placebo	Day 28	Δ PSE-Placebo
Total Cholesterol (mg/dL)	256.0 ± 24.3					
Placebo phase		238.4 ± 18.8	239.3 ± 28.3		241.6 ± 27.2	
Treatment phase		239.9 ± 29.6	237.4 ± 29.2	-1.9 (-1%)	230.4 ± 22.3	-11.2 (-5%)
LDL (mg/dL)	177.1 ± 22.8					
Placebo phase		168.5 ± 22.9	169.8 ± 25.6		169.4 ± 27.0	
Treatment phase		170.8 ± 27.7	157.8 ± 22.8 ^{A, a}	-12 (-7%)	163.3 ± 27.0 ^A	-6.1 (-4%)
HDL (mg/dL)	57.8 ± 19.9					
Placebo phase		50.4 ± 13.8	49.1 ± 13.6		51.2 ± 15.4	
Treatment phase		50.8 ± 15.5	53.3 ± 16.3 ^{A, a}	4.2 (9%)	53.5 ± 16.3 ^a	2.3 (4%)
Triglycerides	125.9 ± 81.4					
Placebo phase		116.6 ± 60.8	122.7 ± 89.0		126.5 ± 74.6	
Treatment phase		111.4 ± 69.8	121.7 ± 71.4	-1 (< -1%)	115.7 ± 63.6	-10.8 (-9%)
Total Cholesterol/HDL	4.89 ± 1.64					
Placebo phase		5.1 ± 1.4	5.2 ± 1.6		5.1 ± 1.6	
Treatment phase		5.1 ± 1.5	4.8 ± 1.6 ^{A, a}	-0.4 (-8%)	4.7 ± 1.4 ^{A, a}	-0.4 (-8%)
LDL/HDL	3.44 ± 1.28					
Placebo phase		3.6 ± 1.3	3.6 ± 1.2		3.7 ± 1.2	
Treatment phase		3.7 ± 1.2	3.4 ± 1.2 ^{A, a}	-0.2 (-6%)	3.3 ± 1.2 ^{A, a}	-0.4 (-10%)
CRP (mg/dL)	0.8 ± 0.5					
Placebo phase		0.7 ± 0.4	0.7 ± 0.4		0.7 ± 0.5	
Treatment phase		0.8 ± 0.6	0.8 ± 0.5	0.07 (5%)	0.7 ± 0.3	0

¹ Values are means ± SD.

² Initial baseline measurement was done 6 week before the placebo phase. Therefore, the data was not included in any analysis, but are provided as reference information

^A Significantly different from placebo, $p < 0.05$ (paired t-test)

^a Significantly different within phase, $p < 0.05$ (1-way repeated measure ANOVA)

cates that regular use of plant sterol esters in a soft gel capsule could contribute significant benefits to long term cholesterol management as the food delivery forms. Previous studies with phytosterol fortified foods have shown reductions of 5% or more in LDL cholesterol levels relative to a control. For example, in men and women with a wide range of age and baseline cholesterol levels, 0.8 g of free sterol equivalents administered as sterol ester in spreads decreased LDL cholesterol by 6% [25], and 1.1 g/d of free sterol equivalents in spreads as plant sterol ester decreased LDL-cholesterol by 4.9% [26]. The significant 4% reduction in plasma LDL-C in the present study indicates that regular use of plant sterol esters is equally effective in a soft gel capsule form compared to food delivery forms of similar doses.

This study was conducted in free-living subjects without collection of dietary records at baseline or during the study. Subjects were advised to maintain regular dietary habits and physical activity. This practical approach was meant to mimic plant sterol capsules consumption under various background diets. Previously, plant sterols were said to be more effective when consumed with diets containing higher levels of cholesterol or fat [2,27], more recent studies indicated that since plant sterols impair both dietary and biliary cholesterol absorption, they are

effective even when consumed with low fat diets [28-30]. Thus, the delivery regimen for the plant sterols is more crucial than the background diet. In the present study, all subjects were advised to take the capsules with each meal (lunch and dinner). As long as plant sterols are taken with meals to stimulate the biliary flow, they can effectively lower cholesterol within the context of various diets and food forms [2].

The present study showed significantly ($p < 0.01$) improved lipid ratios (TC/HDL and LDL/HDL) either independent of placebo or relative to placebo. These results are in agreement with previous reported studies using free stanols [7,8] or sterol esters [31]. The improved lipid ratios are due to reduced LDL, total cholesterol and increased HDL levels during the treatment phase. While most studies published to date have reported that phytosterols have little or no effect on HDL, even with long-term use [5,32], in our study HDL level was increased during the treatment period as compared to placebo ($P < 0.05$). A recent study using orange juice with plant sterols also observed an increase in HDL [33], In studies using plant sterol esters in combination with exercise, significant increase in HDL was observed [34,35]. Since this was a cross-over study and the effect was not observed during placebo phase, it is likely to be a consequence of plant

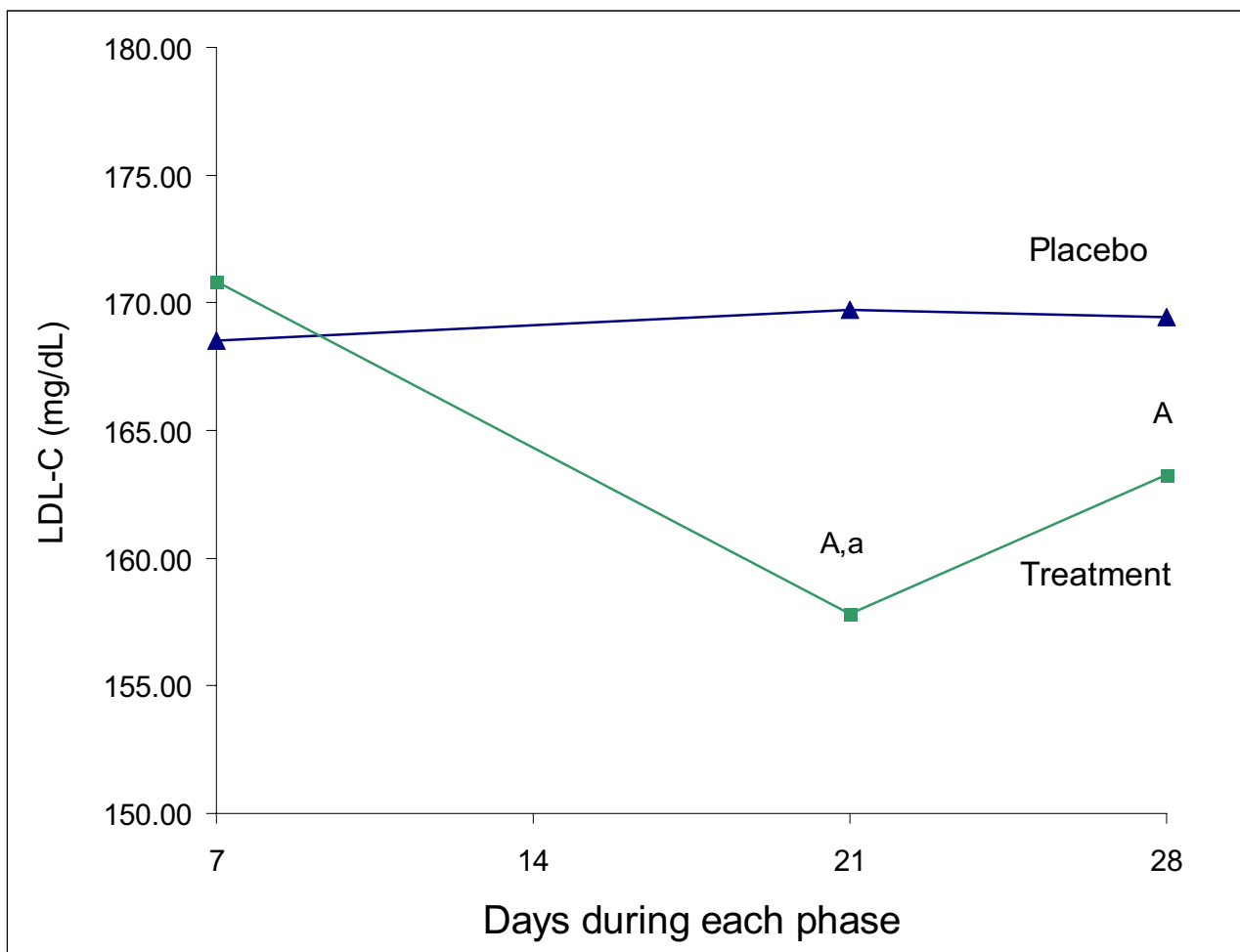


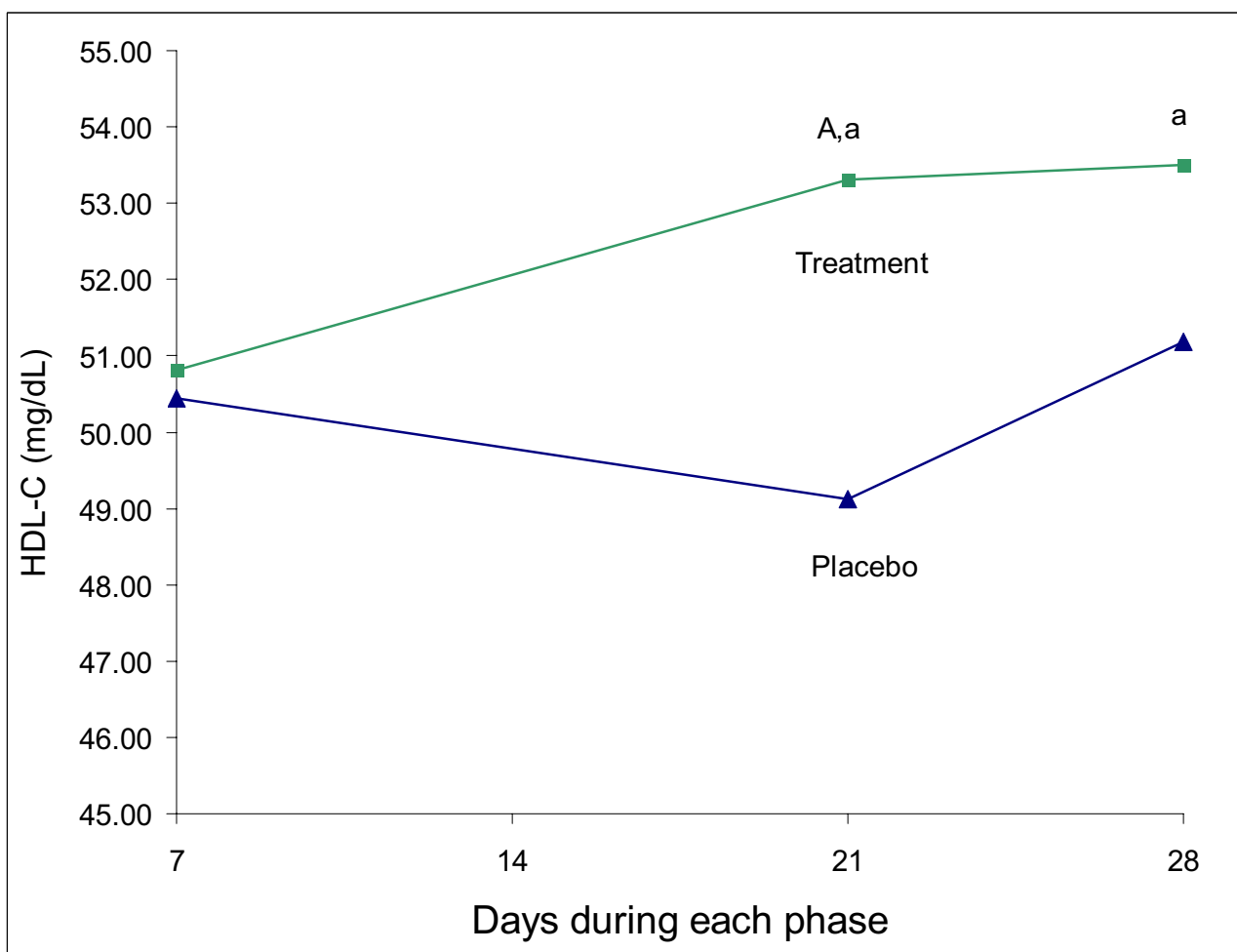
Figure 1

The effect of plant sterol ester on LDL – cholesterol (mg/dL) (N = 16). ^A Significantly different from placebo, $p < 0.05$ (paired t-test) ^a Significantly different within phase, $p < 0.05$ (1-way repeated measure ANOVA)

sterol ester. A longer term administration is needed to further confirm the positive effect of plant sterol ester on HDL levels.

Due to the difference in physical and chemical characteristics between esterized and free forms, plant sterol esters may be a better choice for capsules than their free form. Theoretically, both esters and free forms should possess similar cholesterol lowering effect if the free sterols are properly solubilized. Improperly dispersed formulations, such as aqueous or oil-based suspensions of solid sterols, have been reported as unsuccessful in lowering LDL-cholesterol. These formulations included a crystalline aqueous suspension of sitosterol [36], capsules containing 3 g of stanol powder dispersed in safflower oil [23], or free sterols in beverages with low fat [6]. In addition to the dis-

persion factor, these studies suggested that the crystal form of free sterol/stanols may be another important factor determining their solubility in intestinal fluid and their effectiveness to inhibit cholesterol absorption [37]. To enhance its solubilization in water or oils, the free plant sterols or stanols are usually added to other substances and often require the presence of fat to facilitate the emulsion. Thus, the method of dispersion, processing, and use of emulsifiers, surfactants, and crystal habit modifiers plays a crucial role in delivering small crystals of free phytosterols over time to sufficiently maintain their bioavailability [2,12,13]. A recent breakthrough in formulation, using soy lecithin to form more water dispersible and bioavailable complexes with free stanol, has been demonstrated to lower plasma cholesterol and LDL in two recent studies in tablet form [7,8]. In a placebo controlled,

**Figure 2**

The effect of plant sterol ester on HDL – cholesterol (mg/dL) (N = 16). ^A Significantly different from placebo, $p < 0.05$ (paired t-test) ^a Significantly different within phase, $p < 0.05$ (1-way repeated measure ANOVA)

double-blind study by McPherson et al. [7], free stanols were emulsified with soy lecithin and the spray dried preparation was used to make tablet and capsule forms that were tested in 26 subjects per group over a 6-week period. The group that received tablet form experienced reduction in both LDL cholesterol and LDL/HDL ratio by 10.4% and 11.5% respectively. However, no reduction was observed in the group that received capsules [7]. The author attributed the difference in LDL reduction to the variation in disintegration times, in so far that the tablet disintegrated much faster than the capsule form (10 min. vs. 45 min., respectively).

Fatty acid esters of sterols or stanols are oil soluble. Thus, they are more easily dispersible in oils than free sterols or stanols. Additionally, plant sterol esters are well distrib-

uted in the small intestine providing ample surface area for incorporation into bile salt micelles through the digestive process in order to inhibit cholesterol absorption [2,24]. Earlier evidence suggested that even though the cholesterol lowering effect is similar between free sterols and stanols, the saturation state of their esterified forms may affect the ability to reduce cholesterol absorption [38]. In a clinical study conducted by Jones et al. (2000), intake of plant sterol esters from margarine resulted in larger LDL reductions than margarine with stanol ester (13% vs. 8%, respectively) [38]. However, taking the totality of evidence into account, sterol and stanol esters are probably equal in their cholesterol-lowering effect. Nevertheless, plant sterol esters remain a better choice for soft gel capsules despite the improvements in free sterols formulation to increase the bioavailability.

Finally, this study evaluated the effect of PSE on CRP, a proposed risk factor in cardiovascular disease [39]. All subjects in this study had low or average CRP levels (<3.0 mg/L) at baseline and PSE had no effect on CRP during the treatment phase. It has been demonstrated that diet can have a beneficial impact on CRP levels as observed in the "portfolio diet", a dietary prescription comprised of several foods and food components (including plant sterol esters) which are known to reduce cholesterol levels [40]. In the present study, due to the free-living study design, it is unlikely that CRP will be changed solely based on plant sterol ester treatment.

There are several limitations of this study. First, the study treatments were not randomized. The placebo and PSE treatment were conducted sequentially with wash-out period in between. There is some evidence suggesting a carry-over effect from phytosterol treatment to the placebo group [41] in randomized crossover design. The sequential design could minimize the carry-over effect since the placebo phase was performed first. To minimize the potential confounding effect due to the sequential design, such as time drifting effect, additional within group comparisons were conducted using repeated measure analysis. The results from placebo phase did not show any significant change within the 4-week period whereas significant changes were found within the treatment phase. This within group analyses confirmed that the significant changes were due to the treatment, not the time drifting effect.

Another limitation is the lack of true baseline measurements at the beginning of placebo and treatment phase. This was not intended for the original study design, but occurred during the study. To compensate for this limiting factor, the within group analyses were performed to compare measurements at day 21 and 28 to day 7 of each phase. The significant differences were only detected during the treatment phase, not the placebo phase. Additionally, there was no significant difference at day 7 of each phase between the placebo and treatment suggesting a significant treatment effect.

The number of subjects was calculated based on 10% reduction of total cholesterol. However, given the fact that the tested dose was 1.3 g plant sterol esters/day as 0.8 g free sterol equivalent/day, it is arguable that the above reduction is overestimated. Thus, the study may be underpowered which could explain the lack of significance in reduction of total cholesterol (-5%) ($P = 0.07$). More subjects are needed for further studies with the above dosage. Nevertheless, the present study showed a significant improvement in LDL-C occurred with the present number of subjects indicating that the effect of plant sterol ester is significant.

Conclusion

This study is the first study that confirmed regular use of plant sterol ester capsules is an effective strategy in improving lipid profiles, especially lipoprotein ratios, among hypercholesterolemic subjects in a free-living setting without dietary intervention. The clinical advantage of the capsule form is that it provides a convenient vehicle to consume without the dietary impact on calories. It can also easily be incorporated into a cholesterol-lowering regimen in standard clinical practice with counseling or therapeutic lifestyle changes including those recommended by the National Cholesterol Education Program [24,42]. The significant improvement in LDL-C occurred in free-living subjects with little dietary control indicates that the effect of plant sterol ester is fairly significant regardless of the delivery vehicle and background diet. However, to achieve better lipid lowering effects, higher dosages and incorporation of cholesterol-lowering regimens are recommended. Further studies are needed to confirm the long term benefit of plant sterol ester capsules on blood cholesterol in populations with varying baseline cholesterol levels.

Methods

Subjects

The research protocol and informed consent were approved by the East Tennessee State University (ETSU) Institutional Review Board (IRB) Committee on Protection of Human Subjects.

Subject recruitment was conducted by campus advertisement. The questionnaire evaluation and hematological screening of potential subjects were conducted at the Nutrition Center in the Department of Internal Medicine. Inclusion criteria included men and women (not pregnant or breast feeding), borderline high or elevated serum lipid parameters, body mass index (BMI) < 30, ability to maintain stable diet and physical activity, and in otherwise healthy condition. Candidates were excluded from the study if they had

- diseases or conditions requiring drug intervention;
- been taking cholesterol lowering drugs, supplements or other practices, such as taking cloves of garlic every day
- uncontrolled elevated blood pressure (systolic >160 mmHg or diastolic > 95 mmHg)
- history or presence of drug or alcohol abuse, or alcohol intake >7 alcoholic beverages per week within the past 4 weeks

Power calculations based on expected 10% changes in total cholesterol levels determined that at least 16 subjects

would be needed. Twenty subjects (total cholesterol 227–308 mg/dL, LDL 129–211 mg/dL and triglycerides 43–355 mg/dL) were included in the study. During the study phases, four subjects later withdrew due to either personal reasons or unable following restrictions, such as not taking cholesterol lowering supplements. The remaining sixteen subjects (12 female/4 male, age: 51 ± 13) (total cholesterol: 256 ± 24 mg/dL; LDL: 177 ± 23 mg/dL) completed the study. Table 2 presents the baseline means for the parameters of interest, including age and lipid values, for the 16 subjects who completed the study. Only data from the sixteen subjects were included for analysis.

Materials

Both placebo (containing soybean oil) and matching plant sterol ester capsules were manufactured by Cardinal Health (Dublin, OH). The plant sterol esters (Vegapure® 95) were manufactured by Cognis Corporation (LaGrange, IL). The plant sterol esters were prepared by esterification of free plant sterols from a mixture of soy, rapeseed and other vegetable sources, with fatty acids from sunflower oil. Mixed tocopherols and ascorbyl palmitate are used in the formulation as antioxidants and chemical preservatives. Soybean oil is used as a coating agent and texturizer (see Table 3, Table 4).

Study design and intervention phases

The study was a double-blind, placebo-controlled, non-randomized, sequential study. After the initial baseline measurements which were conducted in late December, there was a six-week period before the placebo phase to minimize lipid profile variance. For this reason, the initial baseline data was not used for comparison to any data collected in placebo and treatment phases

Six weeks after the initial baseline measurement, all subjects were assigned to a placebo phase (soybean oil) for four weeks followed by a two-week, wash-out period before proceeding to a four-week treatment phase. Subjects were instructed to take 1 capsule at each of 2 meals per day (lunch and dinner) during each phase. The PSE

treatment provided a total of 1.3 grams per day of sterol esters (0.8 grams free plant sterol equivalents). This amount is the minimum dose recommended in FDA's health claim for phytosterols [22].

At the beginning of each phase, each subject was given a bottle containing 60 capsules (56 capsules are needed for 4 weeks and 4 capsules are extra). Placebo capsules and plant sterol ester capsules were identical in terms of appearance and sensory characteristics. Compliance was monitored by counting extra capsules in the bottles at the end of each phase and questioning subjects regarding missed doses. Subjects were instructed to maintain their stable diet pattern and physical activity level during the study periods. Although general advice on healthy life style was given to subjects occasionally during their visit to the Nutrition Center, neither dietary advice was given, nor were food records collected.

Fasted blood samples were collected at 7, 21 and 28 days of each phase. The primary measurements were change of plasma total cholesterol (TC), HDL-cholesterol and LDL-cholesterol between placebo and treatment. The secondary measurements were changes of triglycerides, lipoprotein ratios (TC/HDL, LDL/HDL) and CRP.

Lipids and C-Reactive Protein (CRP) analyses

Fasted serum samples were analyzed for triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and c-reactive protein (CRP). Total cholesterol, HDL, triglycerides and CRP were performed at Johnson City Medical Center Hospital (Johnson City, TN) using an automated clinical chemistry analyzer (Beckman Synchro LX20; Fullerton, CA). LDL-cholesterol was calculated using the Friedewald formula.

Statistical analysis

Data were analyzed to detect significant differences between placebo and treatment by ANOVA. Once the significant difference was detected, paired student t-tests was

Table 2: Subject Characteristics at Baseline (n = 16)

Variable	Mean + SD	Range
Age (yrs)	51 ± 13	25–72
Number of Subjects: Female/Male	12/4	
Total Cholesterol (mg/dL)	256.0 ± 24.3	227–308
LDL-Cholesterol (mg/dL)	177.1 ± 22.8	129–211
HDL-Cholesterol (mg/dL)	57.8 ± 19.9	36–110
Triglycerides (mg/dL)	125.9 ± 81.4	43–355
Total Cholesterol/HDL	4.89 ± 1.64	2.25–8.11
LDL-Cholesterol/HDL	3.44 ± 1.28	1.17–5.69
C-Reactive Protein (mg/dL)	0.8 ± 0.5	0.5–1.9

* SD = Standard Deviation

Table 3: Composition of Vegapure® 95

Component	Amount (% W/W)
Mixed Phytosterol Esters and Phytosterols	Min. 97.0%
Ascorbyl Palmitate	250 ppm
Mixed Tocopherols	180 ppm
Soybean Oil	70 ppm
Total	100.0%

performed to compare the measurements at each blood collection time (7 day, 21 day and 28 day) between placebo and treatment group. Additional within-group analysis was performed by 1-way repeated measures analysis of ANOVA to compare measurements within placebo or treatment phase in order to make sure that the expected changes were not caused by time drift during each phase. Paired student t-tests were performed to compare measurements at each blood collection time.

Abbreviations

BMI = body mass index

CHD = coronary heart disease

CRP = c-reactive protein

dL = deciliter

ETSU = East Tennessee State University

FDA = Food and Drug Administration

g = gram

GCP = Good Clinical Practice

GLP = Good Laboratory Practice

GRAS = Generally Recognized as Safe

HDLC = high-density lipoprotein cholesterol

HPLC = High Performance Liquid Chromatography

Hg = mercury

IRB = Institutional Review Board

L = liter

LDLC = low density lipoprotein cholesterol

mg = milligram

NCEP = National Cholesterol Education Program

PSE = plant sterol esters

TC = total cholesterol

TG = triglycerides

Competing interests

All authors have read and approved this manuscript. RVA and ZPD do not have competing interests from the sponsor. DJC and DB are research scientists at Cognis Corp. (USA) and Cognis Deutschland GmbH & Co. KG, respectively, who were involved in interpreting the data and preparing the manuscript, but were not involved in conducting the study.

Authors' contributions

RVA established the study design, obtained study funding and contributed to data interpretation. ZPD recruited subjects, completed instrumental analysis and data collection. DJC wrote the manuscript, conducted statistical

Table 4: Sterol composition of Vegapure® 95 (as free sterols)

Component	Average Percent
β-Sitosterol	49
Campesterol	24
Stigmasterol	17
Brassicasterol	4
Campestanol	3
Sitostanol	1
Other sterols	2

analysis, contributed to data interpretation and final revision. DB contributed to data interpretation and revised the manuscript.

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Review

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Plant sterols: factors affecting their efficacy and safety as functional food ingredients

Alvin Berger¹, Peter JH Jones^{*2} and Suhad S Abumweis²

Address: ¹Head, Biochemical Profiling, Paradigm Genetics, P.O. Box 14528, Research Triangle Park, North Carolina, 27709-4528, USA and ²School of Dietetics and Human Nutrition, McGill University, 21,111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, H9X3V9, Canada

Email: Alvin Berger - aberger@paragen.com; Peter JH Jones* - jonesp@macdonald.mcgill.ca; Suhad S Abumweis - sabumw@po-box.mcgill.ca

* Corresponding author

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Abstract

Plant sterols are naturally occurring molecules that humanity has evolved with. Herein, we have critically evaluated recent literature pertaining to the myriad of factors affecting efficacy and safety of plant sterols in free and esterified forms. We conclude that properly solubilized 4-desmethyl plant sterols, in ester or free form, in reasonable doses (0.8–1.0 g of equivalents per day) and in various vehicles including natural sources, and as part of a healthy diet and lifestyle, are important dietary components for lowering low density lipoprotein (LDL) cholesterol and maintaining good heart health. In addition to their cholesterol lowering properties, plant sterols possess anti-cancer, anti-inflammatory, anti-atherogenicity, and anti-oxidation activities, and should thus be of clinical importance, even for those individuals without elevated LDL cholesterol. The carotenoid lowering effect of plant sterols should be corrected by increasing intake of food that is rich in carotenoids. In pregnant and lactating women and children, further study is needed to verify the dose required to decrease blood cholesterol without affecting fat-soluble vitamins and carotenoid status.

Background

Plant sterols are plant compounds with similar chemical structure and biological functions as cholesterol [1]. Plant sterols contain an extra methyl, ethyl group or double bond. The most abundant plant sterols are sitosterol, campesterol and stigmasterol [2]. The daily dietary intake of plant sterol is 160–400 mg among different populations [3–9]. However, in the earlier stages of human evolution, some 5–7 million years ago, plant sterol intake in Myocene diets would have been considerably higher, up to 1 g/d [10]. Dietary sources include vegetable oils (especially unrefined oils), nuts, seeds and grains [1]. Absorption efficiency for plant sterols in humans is considerably less than that of cholesterol. Percent absorption of the former is 2–5% [11] versus 60% for the latter [12]. Consequently, blood levels of plant sterols in humans are only

0.1–0.14% of cholesterol levels [13]. Due to their structural similarity to cholesterol, plant sterols were first and foremost studied for their cholesterol absorption inhibition properties. In addition to their cholesterol lowering effect, plant sterols may possess anti-cancer [14], anti-atherosclerosis [15,16], anti-inflammation [17] and anti-oxidation activities [18]. The objective of the present review is to assess the evidence supporting the various physiological effects of plant sterols with emphasis on recent advances in knowledge.

Physiological effects of plant sterols Cholesterol lowering actions

The cholesterol lowering effect of plant sterols is well documented in the literature. It is now accepted, after much earlier scientific debate and study, that 4-desmethyl plant

Table 1: Effects of free sterols and stanols on LDL cholesterol and cholesterol absorption parameters.

Reference	Products	Vehicle	Subject	Condition: Starting mean TC mM	Daily Dose g Free sterol/ stanol Equivalents	#Servings/d	Outcome: Where possible, Placebo-adjusted % decrease LDL is given = % decrease with sterol (T_o vs T_f) % decrease with placebo (T_o vs T_p). C-abs also indicated
[59]	A2 C2	Spread	9	Normocholesterol emic	1.0 1.3		↓ C-abs 42.0% ↓ C-abs 33.0%
[176]	B2	Sunflower oil (Capsule)	3 M, 3 W	Hypercholesterole mic	1.5		↓ LDL 14.4%
[27]	A1 B1	Mayonnaise	24 M&W	6.5	0.96 1.0	Habitual usage	↓ TC 5.7% ↓ TC 2.4%
[28]	A1 B1 D1	Mayonnaise	22 M, 9 W	>6.0	1.0 1.0 1.2	Habitual usage	↓ LDL 6.2% ↓ LDL 5.1% ↓ LDL 7.7%
[21]	B1+step 1 diet	Capsules with sterols consumed with food	33 M	6.2	3.0 3.0 with 8 g cholestyramine 8 g of cholestyramine	3	↓ LDL 1.8%
[33]	A2	Spread	12 M	Normocholesterol emic	0.7		↓ LDL 6.1%
[177]	A2	olive oil	8 M, 8 W	3.7 M, 4.1 W	0.4 g/1000 Kcal	3	↓ LDL 2.8%
[103]	A1	Spread	22	5.0 minimum	1.6		↓ LDL 15 % Normocholesterolemics ↓ LDL 4.7% hypercholesterolemics
[97]	B1+prudent diet	Spread	32 M	6.7-6.8	1.7	3	↓ LDL 15%
[22]	B2 +soylecithin	Spread	4 M, 2 W	5.2	1.0-B2 powder 0.3-0.7-B2/lecithin micellar mix		↓ C-abs 11.0% ↓ C-abs 34.4% at 0.3 g ↓ C-abs 36.7% at 0.7 g
[20]	A2	Spread	39 M, 37 W	4.9-5.1	0.8	Habitual usage	↓ LDL 6%, ↓ TC 9%
[69]	B1	Liquid emulsion	12 M	6.9	3	3	↓ C-abs 40%
[100]	A1 B1 1:1 mixture A1:B1	Butter fat ± sterols	9 M, 6 W	6.5	1.8	3	↓ LDL 13.3%, ↓ C-abs 56.1% ↓ LDL 13.4%, ↓ C-abs 33.3% ↓ LDL 16.0%, ↓ C-abs 49.2%
[44]	A2	Vegetable oil, partly filled milk	18 M	7.0	1.8	2	↓ C-abs 39%
[71]	A2	Vegetable oil, partly filled milk	33 M	<5.2	2.2	2	↓ C-abs 51.1%
[42]	A2	Vegetable oil, partly filled milk	18 M, 51 F	7.0	1.2 or 1.6	2	↓ LDL 7.1% at 1.2 g ↓ LDL 9.6% at 1.6 g

Abbreviation: C-abs, cholesterol absorption; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; T_o and T_f refer to start and end points of the study; M, men; W, women; A, free plant sterol; B, free plant stanol; C, plant sterol ester; D, plant stanol ester; 1, tall sterol; 2, soy sterol; 3, shea sterol; 4, corn sterol; 5, rice sterol.

sterols or stanols, either in their free or esterified form, decrease blood levels of total cholesterol and LDL-cholesterol through reduction of cholesterol absorption. Gener-

ally speaking, properly solubilized free sterols and esterified sterols possess similar cholesterol lowering ability [19,20]. In some studies such comparisons have been

Table 2: Effects of esterified sterols and stanols on LDL cholesterol and cholesterol absorption.

Reference	Products	Vehicle	Subject	Condition: Starting mean TC mM(range)	Daily Dose g Free sterol/stanol Equivalents	#Servings /d	Outcome: Where possible, Placebo-adjusted % decrease LDL is given = % decrease with sterol (T ₀ vs T ₁) % decrease with placebo (T ₀ vs T _p)
[32]	D1	Mayonnaise	11 M, 4 W	>6.0	0.8 2.0	Habitual usage	↓ LDL 7.7% ↓ LDL 15.0%
[102]	D1	Spread rapeseed	153 M&W	5.9–5.9	1.8 or 2.6	3	↓ LDL 10.2%
[25]	C2 (66% esterified), D1-2, C3, C5	Spread	100	<8.0	3.3 D1-2 3.3 C2 1.5 C3 1.5 C5	2	↓ LDL 13% ↓ LDL 13% ↔ LDL ↔ LDL
[178]	CD1	Spread	23 W	5.5–8.0	2.4, 3.2, 3.2		↓ LDL 8–10% ↑ HDL 5–6%
[104]	D2 D1-2	Spread, low fat diet	20 M, 35 W	6.1–6.6	2.2 2.3		↓ LDL 13.7% ↓ LDL 8.6%
[104]	C2	Spread	42 M, 58 W	5.2(2.7–7.4)	0.8 1.6 3.2	2	↓ LDL 6.2% ↓ LDL 9.2% ↓ LDL 9.8%
[76]	C2 D1	Spread	34 M&W	4.8–7.0	2.0	2	↓ LDL 10.4% ↓ LDL 12.7%
[37]	D1-2	Spread	14 M, 8 W	6.9(5.0–8.5)	0.8 1.6 2.4 3.0	2–3	↓ LDL 1.7% ↓ LDL 5.6% ↓ LDL 9.7% ↓ LDL 10.4%
[98]	D1-2	Spread	105	6.0–6.6.1	2.0 3.0	2 3	↓ LDL 8.9% ↓ LDL 6.7%
[77]	C1 D1	Spread	15 M	6.4–6.5(6.0–10.0)	1.8 1.8	2–3	↓ LDL 13.4%, ↓ C-abs 36.2% ↓ LDL 6.4%, ↓ C-abs 25.9%
[179]	C2, D1-2	Spread	5 M, 2 W	Ileostomy	1.5		↓ C-abs 38–39%
[99]	D2 D1-2	Spread	41 M, 71 W	5.0–5.1	3.8 4.0	3	↓ LDL 12.6% ↓ LDL 1.6%
[96]	D1-2	Spread	11 M, 28 W	4.9 M, 4.7 W	2.5-different # servings	1 3	↓ LDL 9.9 ↓ LDL 10.2%
[38]	C2	Spread, Step 1 diet	224 M&W	6.2	1.1 2.2	2	↓ LDL 4.9%, ↓ TC 2.6% ↓ LDL 5.4%, ↓ TC 4.0%
[78]	C (mainly 2)	Spread	34 M, 28 W	7.2 (control) 6.5 (treatment)	2.5		↓ LDL 10–15%
[73]	C2	Beef	34 M	5.9	2.4	1	↓ LDL 15%
[70]	D	Yoghurt	16 M, 44 W	5.1	3.0	3	↓ LDL 14%
[65]	C	Spread	25 M, 38 W	6.1	1.8	2	↓ LDL 5.4%, ↓ TC 3.4%

Table 2: Effects of esterified sterols and stanols on LDL cholesterol and cholesterol absorption. (Continued)

[79]	C (vegetable oil)	Spread, Japanese basal diet	26 M, 27 W	5.5	1.8	2	↓ LDL 9.2%, ↓ TC 5.8%
[80]	C (vegetable oil)	Spread	19 M, 31 W	6.0	2.1		↓ LDL 12.3%, ↓ TC 8.9%
[180]	D	Spread	38 M, 22 W	6.1 (2 g group) 6.0 (3 g group)	2.0 3.0	2 3	↓ LDL 9.6%, ↓ TC 6.3% ↓ LDL 7.3%, ↓ TC 5.5%
[71]	C2	Vegetable oil, partly filled milk	33 M	<5.2	2.2	2	↓ C-abs 57.4%

See Table 1 for abbreviations.

flawed because the free sterols were not properly solubilized [21]. Ostlund et al. [22] showed that emulsions of sitostanol, mixed with lecithin containing 0.7 g of sterol, reduced cholesterol absorption considerably, whereas less effect was seen with sitosterol in crystalline form.

This review will focus on the effects of 4-desmethyl sterols, stanols, and esterified forms. Methylated sterols (4 α -monomethyl and 4,4-dimethyl) in sources such as shea and *M. alpina* fungi for example, and those sterols esterified to non-fatty acids such as ferulate (such as the sterols in rice bran oil), may not be equivalent in cholesterol lowering ability compared with the forms present in tall and soybean oils [19,20,23-26].

Important issues that remain to be verified regarding the cholesterol lowering effect of plant sterols includes (i) efficacy of low dose of plant sterols, (ii) the effect diet background on plant sterol efficacy, (iii) the efficacy of plant sterols when incorporated in food other than fat spread (iv) the optimal number of plant sterols servings and (v) the relative efficacy of plant sterols among different populations.

Efficacy of low dose of plant sterols

Tables 1 and 2 summarize recent human intervention clinical trials assessing the effects of 4-desmethyl free and esterified plant sterols. Doses of plant sterols reported in literature are often difficult to comprehend, particularly those reported in earlier literature. Herein, all doses refer to free plant sterol equivalent doses. If the contribution of naturally occurring plant sterols in the food vehicle was reported, this is then added to the free plant sterol dose. Ideally, the free plant sterol dose should be calculated experimentally using the average mol% of fatty acids relative to free sterols.

Selected studies 1990–1994

Vanhanen and Miettinen [27] in 1992 found a dose of 0.95 g of sitosterol per day, including the contribution of free sterols present in the canola oil used to prepare the

basal spread, probably consumed in 2–3 doses, did not result in reduction in total cholesterol compared to control group. LDL cholesterol typically follows changes in total cholesterol, sometimes being more responsive to plant sterol modulation. Since the control spread contained 0.36 g of rape seed oil derived sterols, a level of consumption by vegetarians, the study is essentially comparing vegetarian levels of consumption of plant sterols to a 3-fold higher level. The conclusion from this study alone would be that a higher dose than 0.95 g of free sterols should be considered to achieve a more consistent and effective lowering of LDL cholesterol levels. In another study with a design similar to that of Vanhanen and Miettinen [27], the absolute reduction in LDL cholesterol was only statistically significant for the sitostanol esters, which showed slightly better efficacy than the free sterols and stanols [28]. The dose of 1.0 g sitosterol reduced cholesterol absorption more effectively than the control spread. This is not surprising because absorption is known to be an extremely sensitive marker that does not necessarily correlate to changes in LDL cholesterol levels [7,29-31]. Even basal levels of consumption of plant sterol are correlated with cholesterol absorption.

Vanhanen et al [32] showed that in mildly hypercholesterolemic men and women of age 33–60, 1.2 g of free tall stanol equivalents in mayonnaise decreased LDL levels by 7.7%. Relative to starting levels for this group, this reduction was statistically significant, but the absolute lowering of LDL was not statistically significant after accounting for reductions in LDL cholesterol observed with the control group. The lack of statistical significance in LDL lowering is not surprising because the total sample size was only 15 persons, and there was appreciable plant sterol, about 0.4 g, in the control mayonnaise, which probably reduced LDL cholesterol as well. The quantity of plant sterols in the control spread complicates interpretation of results and makes comparisons to other scientific studies more difficult.

Selected studies 1995–1999

Pelletier et al. [33] demonstrated that 0.7 g of soy sterols in spreads fed to 12 normocholesterolemic individuals reduced LDL cholesterol by 15.2% relative to the control. In another study [20], a dose of 0.8 g of soy sterols fed to 76 normocholesterolemic individuals reduced LDL cholesterol by 6.1% relative to the control, and more importantly, did not reduce carotenoids or carotenoids normalized to cholesterol, as reported by Hendriks et al. [19] with a similar dose of soy free sterol equivalents, administered as an ester. The LDL reductions reported by Sierksma et al. [20] were less than that seen in the Pelletier et al. [33] study, which used a similar dosage. The reduction in LDL in the Seerksma study was not seen in all subjects because of the well-known within person LDL variation of 10% [34] or solubilization issues. Nevertheless, this 6% reduction in LDL correlates with a 15% reduction in CHD risk at age 40, and a 6% reduction at age 70 [35] or a 10% reduction [36].

Hendriks et al [19] showed that in men and women with a wide range of ages and starting total cholesterol from low/normal to high, 0.83 g of free soy sterol equivalents in spreads decreased LDL cholesterol 6.2%. Interestingly, 0.83 g was less effective than the two higher doses, 1.6 and 3.2 g of sterol equivalents, at reducing LDL cholesterol, but the differences amongst the three doses were not statistically significant. This study thus gives strong indication that a 0.8 g free sterol equivalent dose of plant sterols can efficaciously diminish LDL cholesterol. Nevertheless, the authors concluded that the 1.6 g dosage is most desirable because of the lack of effect on lipid normalized carotene, and the quantitatively greater reduction in LDL cholesterol.

Selected studies 2000–2004

Hallikainen et al. [37] showed that in normal to mildly hypercholesterolemic men and women, 0.8 g of free tall/vegetable stanol equivalents in spreads decreased LDL non-significantly by only 1.6%. This 0.8 g dose did reduce the number of apo B particles by 8.7%, indicating a reduced number of LDL particles. Similar to Vanhanen et al. [32], the higher dose of 1.6 g of stanol equivalents reduced LDL cholesterol to a greater extent of 6.1%, and two higher doses (2.4 and 3.2 g/d) reduced LDL cholesterol 10.6–11.5%. The three higher doses (1.6–3.2 g/d) lowered LDL cholesterol in a statistically significant manner. A caveat in this study was that the 0.8 g/d dose was given after cholesterol was already lowered by 3 subsequent plant sterol treatments, possibly producing bias against seeing a reduction in LDL cholesterol with 0.8 g/d. Albeit the above experimental weakness, the conclusion would be that the dose of 1.6 g/d of stanol equivalents is a more optimal dose for LDL cholesterol reduction.

Maki et al. [38] administered mildly hypercholesterolemic men and women 1.1 g/d of free tall sterol equivalents in spreads, fed as sterol esters in two doses, which decreased LDL levels by 4.9% while 2.2 g/d of sterol equivalents decreased LDL by 5.4%.

Christiansen et al. [39] reported an 11.3% reduction in LDL cholesterol after 6 months with 1.5 g/d of microcrystalline free sterols in spreads. No additional improvement was seen with 3.0 g/d of plant sterol.

Volpe et al. [40] reported a 6.3% placebo-adjusted decrease in LDL cholesterol after 4 wks with 1.0 g/d and a greater reduction of about 12.2% with 2 g/d after 4 wks. De Graaf et al. [105] found an intake of 1.8 g/d of free sterols in chocolates to decrease LDL cholesterol 8.9% relative to baseline.

Thomsen et al. [42] examined effects of non-esterified, non-hydrogenated, soy bean derived plant sterols, solubilized in a partly vegetable oil filled low fat milk on serum LDL cholesterol in 81 mildly hypercholesterolemic Danish patients, in a double-blind, randomised, placebo-controlled 3-arm cross-over study. Subjects consumed habitual diets, with some restrictions on consumption of fat and cholesterol rich foods. Subjects received 0, 1.2, or 1.6 g/d of sterols in two servings of 250 mL milk for 12 wks (4 wks/dose). The placebo-adjusted mean reduction in LDL was 7.1 ± 12.3 and $9.6 \pm 12.4\%$ (mean \pm SD) for groups receiving 1.2 and 1.6 g of plant sterols, respectively, with no differences between sexes. There was no statistically significant difference in LDL lowering amongst the 1.2 and 1.6 g/d groups, although Apo B was decreased more with 1.6 than 1.2 g/d of sterols. Apo B is an index of LDL particle number, thus the higher dose may have decreased numbers of LDL particles more than the lower dose. Differences in numbers of small, dense, atherogenic LDL particles and LDL oxidation [43] are other important future parameters to assess. It is noteworthy that there were 20–23% non-responders in the two sterol groups, which was partially consistent with the large differences in cholesterol absorption inhibition observed with similar milk products containing plant sterols [44]. Thus, renewed attention should be given to the issue of non-responders. Another noteworthy observation was the randomization order in which the three milk products affected the magnitude of the LDL lowering results, but not the overall statistical findings. The placebo-adjusted mean percentage decrease in LDL was more pronounced with certain randomisation sequences compared to others. This consideration is typically ignored in reporting results of plant sterol clinical trials examining cholesterol lowering efficacy.

In a very recent study still in press [45], 72 men and women aged 20–73 received two 8 ounce servings of Minute Maid brand non-fat orange juice with breakfast and dinner meals, providing 2 g/d of Cargill CoroWise plant sterols for 8 wks. LDL was reduced 12.4% compared to baseline and placebo; HDL and triacylglycerol levels were not changed. The authors speculate that the fat in the meals may help to emulsify the plant sterols in the orange juice.

Effects of naturally occurring plant sterols

The effects of naturally-occurring plant sterols on cholesterol metabolism have also been studied in both older and more recent literature. It was reported that the differences between effects of different plant oils on blood lipid profiles may be related to their content of plant sterols [46-49]. Indeed, there has been renewed interest in the cholesterol lowering properties of speciality grains and unprocessed oils rich in plant sterols including amaranth oil [50,51], rice bran oil [52] (Berger et al., submitted), avocado oil [53], extra virgin olive oil [54], macadamian nut [55], and argan oil [56].

Ostlund et al. [49] showed that doses as low as 150–300 mg of naturally present corn oil-derived phytosterols can reduce dietary cholesterol absorption. Also, it was shown that the consumption of original wheat germ, which contains about 328 mg plant sterols, reduced the cholesterol absorption by 42.8 % compared to plant sterol-free wheat germ [57]. These results indicate that naturally available plant sterols are biologically effective as plant sterol supplementation in reducing cholesterol absorption, and that natural plant sterols have important effects on cholesterol metabolism [57].

Summary of biologically active dose of plant sterols for optimal cholesterol lowering

Several studies [19,20,28,32,33,40,58] using intakes of 800–1000 mg of plant sterols per day have shown biologically/clinically significant (5% or more) reductions in LDL cholesterol levels, relative to control, or at least showed a statistically significant treatment effect relative to the starting LDL cholesterol level at the beginning of the treatment period, independent of control. Other studies [27,37] with a similar dosage range did not meet the above criteria for biological reduction of LDL levels, or achieve statistical significance. Some studies showed that 800–1000 mg/d of free plant sterol equivalents can decrease the absorption of cholesterol, which is indicative, but not necessarily predictive, of actual LDL cholesterol lowering [22,28,32,59].

It has been shown that increasing the dosage beyond 1000 mg per day of free sterol equivalents increased LDL cholesterol lowering efficacy or consistency of response lead-

ing to more statistically significant results [32,37,40]. Increasing the dosage beyond 1000 mg per day of free sterol equivalents did not further increase LDL cholesterol lowering efficacy [19].

In humans, there is a good likelihood that a dose of 0.8–1.0 g of free sterol equivalents per day, properly solubilized, administered in 2–3 servings with a meal, will reduce LDL cholesterol by 5% or more and that this reduction in LDL cholesterol will correlate with an approximate 6–10% reduction in CHD risk at age 70 [35,36]. However, at this dosage level, it is likely that not all individuals will achieve a 5% reduction in LDL cholesterol [20].

Clinical relevance of LDL-cholesterol-lowering by plant sterols

As previously noted, it is generally agreed that high blood cholesterol level (especially LDL cholesterol) is a risk factor for coronary heart disease (CHD). Oxidation of excess LDL cholesterol leads to arterial wall plaque build up, which then restricts blood flow and increases blood pressure. Unless, hypercholesterolemia and hypertension are treated, these factors are associated with increased risk of coronary heart disease (myocardial infarction) and stroke [35].

Therefore, the clinical relevance of LDL-cholesterol lowering lies in the potential for plant sterols to reduce the actual risk of CHD. As already described, there is an impressive body of scientific data demonstrating cholesterol-lowering by plant sterols. However, it is pertinent to tease out from published studies, those providing the highest level of evidence for a clinically-important effect. Two reviews have addressed this issue [60,61]. Law [60] estimated that consumption of 2 g of equivalents of plant sterol or stanol per day would reduce heart disease risk 25%. But only a randomized clinical trial using CHD as an endpoint, could provide certainty of the effectiveness of plant sterols in reducing heart disease incidence. But for a clinical trial to detect a 12–20% reduction in coronary heart disease incidence would require 10,000–15,000 patients with CHD (and more for healthy people). Even if such a trial were feasible, it would probably still be underpowered to detect any rare adverse events (undesirable side effects) [61]. Thus, we must judge the effectiveness of plant sterol doses on their theorized ability to reduce CHD incidence, using LDL cholesterol as a marker.

Low fat versus high fat background diet

Dietary cholesterol consumption is 250–500 mg/d, and normally half is absorbed, while biliary cholesterol production is 600–1000 mg/d. Since plant sterols impair the absorption of both biliary and dietary cholesterol, it is not surprising that they are effective even when consumed in low fat diets [62,63], although evidence from some studies suggests them to be more effective when consumed

with diets containing cholesterol [21,64,65]. In a study by Denke [21], plant stanols were given in capsules and not blended with fatty matrix, which limits their cholesterol-lowering action. In addition, compliance was monitored by capsule counting and not by direct supervision, which decreased compliance monitoring. Mussner et al. [65] found esterified phytosterols in spreads to reduce LDL cholesterol about 5.4%, but the reduction was 11.6% in those tertiles having the highest intake of dietary cholesterol. Recent studies have shown plant sterols to be effective even if consumed with Step I diets [38,40,66]. Similarly, Judd et al. [67] showed that high doses of vegetable oil sterol esters lowered LDL cholesterol to about the same level, whether the basal diet was a typical American diet or a Step I type of diet, suggesting dramatic changes in usual fat intake are not necessary, if plant sterols are consumed concurrently.

Vehicle for delivering plant sterol

Most clinical trials have been conducted using plant sterols or stanols added to spreads. As long as plant sterols are consumed with a meal to stimulate biliary flow, they can effectively lower LDL cholesterol on the background of various types of basal diets and food vehicles. Plant sterols are efficacious when consumed in: oil: water emulsions [68,69]; water as lecithin micelles [22]; yogurt [40,70]; low fat filled milks [42,44,71]; chocolate [105]; cereal; snack bars, breads, and beverages [66,72]; and beef/hamburger [73,74]. Efficacy of a soy stanol-lecithin powder in reducing cholesterol absorption and LDL-C has been evaluated in a randomized, double-blind parallel study [75]. The subjects who followed a Step I diet consumed soy stanol-lecithin powder in a beverage. The provided daily dose of plant stanols was 1.9 g. The reductions in blood cholesterol and LDL cholesterol were 10.1 and 14.4%, respectively. In another group of subjects, cholesterol absorption was measured using 625 mg stanols provided in beverage or egg whites. Stanol-lecithin reduced cholesterol absorption by 32.1% and 38.2 % when consumed in beverage and egg white, respectively.

The reduction in LDL cholesterol reported, using the previous different vehicles, ranged between 7–14 %, which is close to the reduction in LDL cholesterol reported in studies that used fat spread as a vehicle for delivering plant sterol [25,37,76-82]. A recent controlled clinical trial has shown that the intake of plant sterols provided in low-fat and non-fat beverages did not affect lipid profiles in moderately hypercholesterolemic individuals [83]. The finding of this study was contrary to the findings of other studies reporting that plant sterols were effective in reducing blood cholesterol even when incorporated into low-fat or non-fat foods [40,42,44,70,72]. This discrepancy may be related to the fact that the plant sterols must be added to the low or non fat food matrix in such a way that

the plant sterols solubilize, or remain as small crystals over time. In products such as milk, the milk fat globule membrane components may enhance the absorption of cholesterol [44,68,69], but also aid in solubilization. Pouteau et al. [44] described a rapid filtration and detection method to quantify plant sterol crystals. The authors also used light scattering techniques to quantify the size of the crystals as a function of storage time of the milk products. The method of dispersion, processing, and use of emulsifiers, surfactants, and crystal habit modifiers will affect the success of plant sterols in non-spread vehicles [42,44,71,84,85].

Commercially, plant sterols are currently contained in bars (Logicol-Australia, Benecol-UK), vegetable oils (Ekona-Japan; NutraLease Canola Active-Israel), orange juice (Minute Maid Heart Wise containing Cargill CoroWise plant sterols) [45], mayonnaises (Logicol-Australia), milk (Benecol-UK, Logicol-Australia, SereCol-Argentina), yogurt (Logicol-Australia; Benecol-UK), yogurt drinks (Benecol), soy milk (Pacific Foods), meat and soups (Raisio-Finland), and green teas (Chol zero, Korea). Plant sterols are also being sold or developed mixed with other functional ingredients such as: fiber (Unilever Fruit D'or-France); healthy oils (Benecol Olive Spread-UK); non-absorbable diacylglycerol (Kao-ADM Econa Healthy Cooking Oil; Enzymotec MultOil Platform, ArteriCare products, Israel); almonds, soy protein and viscous fibers [86]; and minerals [87-89]. There is also interest to combine plant sterols with antioxidants, such as flavonoids, quercetins, and catechin; and a spice mixture developed by Selako, and marketed as Flavomare in Scandanavia. It is only a matter of time before ingredients such as conjugated linoleic acid (CLA) are mixed with plant sterols in various vehicles (*e.g.*, Clarinol's CLA has received GRAS status for addition to milks, yogurts, bars, etc.).

Various manufacturers also sell plant sterols in supplement form, and there is interest to develop plant sterols as drugs (*e.g.*, Forbes' FM-VP4 drug candidate). Plant sterols may also be combined with other drugs that lower cholesterol through different mechanisms of action, including statins and ezetimibe [90,91]. Recent evidence suggests that patients who had previous acute coronary syndrome benefited from aggressive LDL lowering with statins to levels substantially below current target levels [92,93]. This finding provides enthusiasm for developing novel plant sterol-drug and drug-drug combined strategies to aggressively lower LDL cholesterol in some populations. Despite evidence that plant sterols can effectively reduce LDL cholesterol and inhibit cholesterol absorption in vehicles other than spread type vehicles, regulatory agencies have been slow to accept plant sterols in foods other than spreads in some countries such as the USA [94] and Australia [95]. Rigorous efforts underway by food

companies and other highly respected organizations to allow claims for plant sterols in foods other than spreads.

Optimal number of servings

It has been suggested that plant sterols should be consumed at each cholesterol containing meal to achieve an optimal effect. A daily intake of 2.5 g plant stanol esters, either consumed once per day at lunch, or divided over three portions resulted in a similar decrease in serum total and LDL cholesterol levels [96]. Similar efficacy with a single larger dose sterol esters has also been demonstrated in two additional studies [73]. A single serving of yogurt, providing 1 g of pure free sterols, resulted in a placebo-adjusted reduction in LDL cholesterol of 6.3% [40]. Consumption of a single dose of 2.4 g/d plant sterols resulted in a 9.3 and 14.6 % reductions in blood total and LDL cholesterol levels, respectively, in hypercholesterolemic individuals [73]. Single doses of plant sterols may have sustained effects on cholesterol absorption via interactions with intestinal proteins (see Section 3.1.5.1 for details).

Nevertheless, as there are a plethora of studies showing the efficacy of plant sterols distributed in 2–3 meals [19,25,37,38,70,76-82,97-100], and only two studies to date demonstrating efficacy with a single larger serving [73,96], it seems prudent to remain consistent with the more established, conservative recommendation of consuming plant sterols in 2–3 doses with food, as adopted by the United States FDA.

Population under study

Plant sterol for the adult population

Typically, cholesterol lowering properties of plant sterols are similar in both men and women, although recent studies highlight that plant sterols can diminish fat soluble vitamins only in women [37]. Mixed gender studies must possess the statistical power to separate men and women as a statistical covariant, otherwise, the researcher must assume an identical response across both sexes.

The recent study of Matvienko et al. [73] demonstrates that soy sterol esters can effectively decrease LDL cholesterol in young adults of age 23, suggesting age is not a very critical variable influencing LDL cholesterol lowering properties of plant sterols, as also confirmed in studies with children [101]. In contrast, the meta-analysis of Law [60] predicted that plant sterol and stanol esters would reduce LDL cholesterol more effectively at each dose in older compared with younger people. However, it should be taken into consideration that older people had higher starting circulating lipid levels, so the percent change did not differ across age ranges. A number of studies have shown that plant sterols effectively reduce blood cholesterol in normocholesterolemic

[19,22,25,37,59,76,96,102,103], hypercholesterolemic subjects [37,38,40,62,72,73,76,77,80,97,104-106], subjects with familial hypercholesterolemia [78,100], and in type II diabetic hypercholesterolemic patients [107,108]. Further, in a type II diabetic population consuming statin, plant sterols had a combined effect on lowering LDL cholesterol an additional 27%, the combined effect being 44% [108]. The reduction in LDL cholesterol seems to be greater in hypercholesterolemic individuals with type II diabetes. Plant sterols decreased LDL cholesterol in hypercholesterolemic individuals with and without type II diabetes by 14.9 % and 29.8 %, respectively (Lau et al. unpublished data).

Plant sterols are not recommended for pregnant or lactating women. However, there has not been a systematic study testing this issue. Vegetarian women habitually consume up to 500 mg of plant sterols per day. There is no evidence that such women cannot have normal pregnancies. Certain ethnic groups are known to have high levels of plant sterol intake and their pregnancy outcome could be evaluated in future studies. For example, in 372 semi-acclimated Tarahumara Indians in the Sierra Madre Occidental Mountains of Mexico, the diet was found to be high in fiber and to contain less than 100 mg/day of cholesterol and over 400 mg/day of plant sterols [4]. Further, in the earlier stages of human evolution, some 5–7 million years ago, plant sterol intake in Myocene diets would have been considerably higher, up to 1 g/d [10]. Such diets were not only rich in plant sterols, but also dietary fiber, vegetable protein, and associated phytochemicals; but low in saturated and trans-fatty acids [10]. To meet the body's needs for cholesterol, genetic differences and polymorphisms were conserved by evolution, tending to raise serum cholesterol levels.

Plant sterols likely interact with ATP-binding cassette (ABC) transport proteins to direct cholesterol back into the intestinal lumen, regulating absorption of cholesterol and plant sterols [109-113]. Plat and Mensink [114] first hypothesized that plant sterols increased the expression of ABCA1. Thereafter, based on an animal study, it was suggested that plant sterols are converted into a liver X receptor (LXR) agonist, which activates the expression of ABC proteins [115]. Mutations in ABC proteins are responsible for the rare disease sitosterolemia [116]; and polymorphisms of ABC proteins may affect cholesterol absorption based on a preliminary study [117]. Polymorphism of ABCG8 gene was found to contribute to blood plant sterol levels in healthy subjects [118] suggesting ABCG8 protein regulates non-cholesterol sterol absorption.

Apolipoprotein E phenotype was originally shown to be correlated with cholesterol absorption [119]. It was

shown in one study [120] but not others [37,99] to affect plant sterol cholesterol lowering efficacy in recent trials.

In addition to the above proteins, cholesterol absorption is likely controlled by additional proteins [121], as well as a putative sterol transporter system [122]. In this context, the genotype of apolipoprotein A-IV, scavenger receptor-BI, 3-hydroxy-3-methyl-coenzyme A reductase, apolipoprotein E, and cholesterol ester transfer did not affect cholesterol lowering effects of plant stanol [122].

Plant sterols for children

Plant sterols are not recommended for normocholesterolemic children under five because children who are growing have a large need for cholesterol for normal development. There is also the fear that plant sterols, particularly esters, could affect the absorption of fat soluble vitamins. However, no direct evidence points at plant sterols being in some way dangerous for children. Studies with small amounts of plant sterols fed to infants have shown that neonates have the adaptive ability to increase their cholesterol synthesis [123-125]. In fact, infants are typically fed formula diets containing cholesterol concentrations 3–35 times lower than breast milk, with considerably higher levels of plant sterols [126]. There is the possibility that cholesterol, received in utero or administered to neonates, could affect gene expression and physiology later in life. This theory was initially based on the increased atherosclerosis incidence in adults fed formula rather than breast milk as infants [127], as well as higher cholesterol in men fed breast milk for less than 3 months as compared to more than 9 months [128]. This so called "cholesterol imprinting" hypothesis is now being explored in controlled animal models with microarrays [129]. Children with allergies to dairy routinely consume vegetable oils rich in plant sterols and less cholesterol, and thus have less cholesterol absorbed, but compensatory increases in cholesterol synthesis [130].

Most studies examining the effects of plant sterols in children have been conducted with hypercholesterolemic children [131-134]. Generally, plant sterols seem to be as effective in hypercholesterolemic children as in hypercholesterolemic adults. Some older studies in children must be interpreted with caution, as the preparations may have been crystalline [135]. Becker [132], for example, found that severely hypercholesterolemic children could be effectively treated with sitosterol, and that 3 g/d sitosterol combined with a half dose of bezafibrate was an effective way to reduce the bezafibrate dose. Intake of 1.7 g/d of plant sterols in ester form was effective in reducing total cholesterol levels, LDL cholesterol and apo B levels in children with familial hypercholesterolemia who followed Step I diet without any adverse effects [136]. No changes in concentration of lipid-adjusted carotenoids

were reported except for lycopene, which decreased by 8.1%. This decrease was considered of minor biological and clinical importance [136]. The authors recommended an increase in the intake of fruit and vegetables to avoid the reduction in lycopene values when plant sterols were introduced to Step I diet of children with familial hypercholesterolemia.

In a crossover study, healthy 2–5 year old children consumed either 3 g/d plant stanol ester or 5 g/d insoluble wheat bran fiber for 2 weeks, then 10 g/d for the second two weeks [101]. Relative to baseline, LDL cholesterol levels were reduced 15.5% with stanol esters and 4% with the fiber diet. Stanol esters did not affect triacylglycerols or HDL cholesterol. The study showed that stanol esters reduced LDL cholesterol in normocholesterolemic children similarly to that found in normocholesterolemic adults and hypercholesterolemic adults and children. In healthy 6-year-old children who were on a low-saturated, low-cholesterol diet, daily intake of 1.5 g/d of plant stanol ester was effective in reducing total cholesterol and LDL cholesterol values by 5.4% and 7.5%, respectively [137]. The intake of plant stanol did not cause any adverse clinical effects, nor did it affect the levels of fat soluble vitamins; however, it did cause a 19% reduction in ratio of β -carotene to LDL cholesterol ratio.

Children consuming vegetable oil sterols in margarine for 13 months had serum concentrations of campesterol and sitosterol that were 75% and 44% higher than those in the control children, while serum cholesterol precursor sterol concentrations, indicative of cholesterol synthesis, did not differ between the two groups [138]. Thus, doubling dietary plant sterol intake almost doubles serum plant sterol concentrations in 13-mo-old children, but has no effect on endogenous cholesterol synthesis. Relative intestinal absorption of natural plant sterols from the diet in early childhood is similar to that in adults. In the older study of Mellies et al. [139], 300–900 mg/d of plant sterols led to a large accumulation of plant sterols in the plasma (0.44 mM) of normo and hypercholesterolemic children.

As in adults, in children, the apo E phenotype, could be a factor affecting the efficacy of plant sterols. Plant sterols, as an index of cholesterol absorption, were higher in adults or children with the E4/3 phenotype as compared with those with other phenotypes [140]. Lathosterol, an index of cholesterol synthesis, was also higher in children with E4/3 phenotype than in those with E3/3 or E3/2, indicating these children both absorb and synthesize more cholesterol [140]. The effect of phenotype of apo E on response to sterol intake was investigated in 6-year-old children [141]. Daily intake of 1.6 g of plant stanol was effective in reducing blood cholesterol and LDL

cholesterol by 65 and 8%, respectively, in these children regardless of apo E phenotype. Thus, children with different apo E phenotype can achieve a reduction in their cholesterol levels by intake of plant sterol.

From the previous studies, it is clear that plant sterols are effective in reducing blood cholesterol in healthy as well as in hypercholesterolemic children. The only side effect reported is a reduction in levels in ratio of β -carotene to LDL cholesterol ratio and lycopene values, which could be balanced by increasing the intake of fruit and vegetable, especially those rich in carotenoids, as was the case in adult population [106].

Plant sterol intake and sitosterolemia

Sitosterolemia is a rare autosomal recessively inherited disorder which results from absorption of high amounts of plant sterol and cholesterol for unclear reasons linked to a locus at chromosome 2p21 [142-144] leading to development of coronary heart disease at young age, and development of tendon xanthomatosis. Various candidate genes involved in cholesterol absorption have been excluded at present [145]. Sitosterolemic persons should avoid food products containing plant sterols. Hydrogenated plant sterols may be safer than non-hydrogenated plant sterols for this population because the former is less absorbed, however, this argument is speculative. A recent study found that heterozygous subjects for sitosterolemia who received sterol esters in a spread providing 3.3 g of free sterol equivalents for 4 weeks, had a 10.6% reduction in LDL cholesterol [146]. Levels of campesterol and sitosterol were increased, but the magnitude of the increase was not much greater than that observed in normal subjects consuming similar spreads. In another recent study in 12 subjects who were obligate heterozygotes for sitosterolemia, consumption of plant sterol ester for 6 weeks resulted in an additional significant reduction of 5.9% in LDL cholesterol over that provided by a Step I diet alone, but no additional significant reduction was found after consumption of plant sterol ester for 12 weeks [147]. Although plasma levels of plant sterols concentration were elevated, the increase was similar to that reported in normal and mildly hypercholesterolemic subjects who consumed plant sterol esters [147]. The increase in plasma levels of plant sterols reached a plateau, which indicates that obligate heterozygotes eliminated the plant sterols from their body in order to prevent their accumulation. For prudence, it is nevertheless recommended that persons with sitosterolemia avoid plant sterols.

Anti-atherogenicity activity

In vitro studies have shown that plant sterols are effective in preventing hyperproliferation of vascular smooth muscle cell that play a role in atherosclerosis development [148]. Animal studies have shown that plant sterols also

have anti-atherogenicity activity. In rabbits, sitostanol feeding decreased plaque accumulation in coronary arteries within the ascending aorta [149]. Feeding plant sterols to apo E-deficient mice decreased platelet counts as well as the susceptibility of red blood cells to hemolysis, decreased plasma fibrinogen [16], and decreased formation of atherosclerotic lesions [15,16,150]. In healthy subjects who consumed 4 g/d of wood based stanol ester, the activity of antithrombin-III tended to increase compared to control group [99]. Thus, plant sterols may reduce atherosclerosis development not only by reducing blood cholesterol levels but also by possessing anti-atherogenicity activity.

Anti-cancer activity

The action of plant sterols as anticancer dietary components has been recently extensively reviewed [151]. Plant sterols can suppress tumor cell growth (LNCaP and HT-29) [152,153]. Compared to cholesterol, β -sitosterol caused a 24% decrease in cell growth and a 4-fold increase in apoptosis. In the latter work, the authors were interested in the effects of β -sitosterol on the sphingomyelin cycle, and measured two key enzymes: protein phosphatase 2A (PP 2A) and phospholipase D (PLD). A 50% increase was observed in PP 2A activity in media containing 16 μ M of β -sitosterol; however, there were no changes in protein levels of PP 2A. PLD activity increased in presence of phorbol myristate and β -sitosterol. This study suggests that the sphingomyelin cycle, which increases cell apoptosis, is mediated by PLD, PP 2A, and possibly, incorporation of β -sitosterol into the membrane. Another possible mechanism by which β -sitosterol can protect against cancer is through down-regulation of cholesterol synthesis, as was found in MDA-MB-231 human breast cancer cells [14]. In an important *in vivo* study, SCID mice were xenografted with the human breast cancer cell line MDA-MB-231 [154]. Plant sterol-fed mice had a 33% smaller tumor size and 20% less metastases in lymph nodes and lungs than cholesterol-fed mice. This finding implied the possibility that plant sterols may retard the growth and spread of breast cancer cells. In addition to retarding the growth of breast cancer cells by plant sterols, there is some evidence that plant sterols can affect the development of prostate cancer [155]. In a meta-analysis, 519 men were studied in 4 randomized, placebo-controlled, double-blind trials. β -sitosterol improved urinary symptom scores and flow measures, suggesting that non-glucosidic forms of β -sitosterol improve urinary symptoms and flow measures. Long term effectiveness, safety, and ability to prevent benign prostatic hyperplasia complications are not known [155]. In another recent study, there was no evidence that plant sterol usage at dose of 300 mg/d, decreased risk of colon and rectal cancers [156]. A similar conclusion was reached following a rat study in which rats

were given the carcinogen methyl-nitroso-urea and then monitored for tumor development [157].

Plant sterols have also been found to have a protective effect against lung cancer [158]. In this study, intake of about 144 mg/d of plant sterols was associated with reduction in risk for lung cancer even after controlling of confounding factors, i.e. tobacco smoking, vegetables, fruits, and antioxidant substances. Total dietary plant sterol intake was found to be inversely associated with breast [159]), stomach [160], and esophageal [161] cancers. It was found that women with highest quartiles of total dietary intakes of plant sterols (>122 mg/d) had reduced risk of endometrial cancer [162], and intake of more than 521 mg/d reduced risk of ovarian cancer [163]. On the other hand, in a prospective epidemiological study, high dietary intake was not associated with reduced risk of colon and rectal cancers [156]. However, the intake of plant sterol might reduce the risk of more than one type of cancer.

Anti-inflammation activity of plant sterols

Bouic [17] and Bouic et al [164] have reviewed the possible roles of phytosterols in the etiology or preventive role of phytosterols in various diseases and conditions, including proliferative responses of lymphocytes, pulmonary tuberculosis, feline immunodeficiency virus and HIV, stress induced immune suppression, rheumatoid arthritis, and allergic rhinitis/sinusitis. The mechanisms by which plant sterols display their anti-inflammatory activity are thought to include inhibition of secretion of inflammatory mediators such as interleukin-6, and tumor necrosis factor- α by monocytes [17]. Most of the work has been conducted with animals. From these provocative results, it is not unlikely that plant sterols will be further used for purposes related to control of development and spread of certain cancers in humans.

Anti-oxidant activity

Another possible effect of plant sterols is their antioxidant activity [165]. It was found that the methanol extract of soybean oil, which has a strong in-vitro protective effect against DNA damage in human endothelial cell, contains phytosterols in addition to tocopherols and n-3 polyunsaturated fatty acids (PUFA). Results suggest that the antioxidant activity of soybean oil may be in part related to sterol content. Moreover, in in-vitro conditions, sitosterol, and sitosterol glucoside were found to decrease lipid peroxidation of platelet membranes in the presence of iron [18] and in healthy human subjects a 2 and 3-g dose of stanol ester reduced oxidized LDL-C levels [82]. The authors suggested that the intake of stanol ester might protect LDL particles from oxidation. Thus, based on results from in vitro studies and on human study, there is a possibility that plant sterols may possess antioxidant

properties. Such antioxidant protection could also benefit atherosclerosis [166] and cancer [167] disease state.

Anti-ulcer activity

In a recent study, phytosterol esters, but not sterols, in horse gram (an herb in the genus *Dolichos* cultivated in India for food and fodder) were protective in a pyloric ligation model of ulcer, whereas sterols were protective in acute ulcer models using ethanol-induced and cysteamine-induced ulceration [168]. Phospholipids were protective in both types of model. Thus, the presence of sterols, sterol esters, and phospholipids in food lipids in staple diets may account for the low prevalence of duodenal ulcer in certain geographical areas, despite a uniformly high prevalence of *Helicobacter pylori* infection.

Anti-fungal activity

Another area for future investigation is the anti-fungal activity of plant sterols and related triterpenes [169]. In this work, the anti-fungal activity of triterpenes in the mushroom species *Ganoderma annulare* was demonstrated.

Safety

It has been concluded that plant sterols, within the range that causes desirable reduction in blood levels of total cholesterol and LDL-cholesterol, are clinically safe. This conclusion has been reported in short-term studies [19,39,40,170] as well as in long-term study that lasted for 1 year [81]. Since plant sterols decrease the absorption of cholesterol, they might also affect the absorption of fat-soluble vitamins. The scientific evidence for the impact of phytosterols on carotenoid status and fat soluble vitamins is summarized in Table 3. The effect of plant sterols on the blood levels of precursors of fat-soluble vitamins is a controversial issue. In some studies, plant sterols consumption has been shown to significantly reduce levels of carotenoids [25,37,38,81,170,171], tocopherol [37], and lycopene [25,38]. Other studies reported that the consumption of plant sterols does not affect blood levels of carotenoids [39,72,104,172], tocopherol [19,39,173], and lycopene [19,173].

In a recent trial comparing equal free sterol equivalent amounts (2.2 g/d) of esterified sterols and free sterols in milk, both forms of sterols decreased the absorption of β -carotene and α -tocopherol in normocholesterolemic men. The reduction in β -carotene bioavailability was significantly less pronounced with free plant sterols than with plant sterol esters. However, there was no difference in cholesterol absorption between the two forms of plant sterols. [71]. Esters are presumed to have more of an effect on fat soluble vitamins because they partition into the oil phase of the intestine, whereas free sterol would partition into the micellar phase [174].

Table 3: Summary of scientific evidence for the impact of phytosterols on carotenoid status and fat soluble vitamins

Reference	Products	Vehicle	Subject Mean Age or range	Condition: Starting mean TC mM (range)	Dose g/day (free sterol/ stanol equivalents)	Duration of study wks	Outcome	
							Impact of phytosterols on vitamin A and carotenoid status	Impact of phytosterols on other fat soluble vitamins
[25]	C, D2, D3, D5	Spread	Adults, 18–65	<8	1.5–3.3	3.5	↓ absolute and lipid standardized levels of α - and β -carotene and lycopene.	
[178]	D1	Spread, Butter	Postmenopausal women aged 50–55	5.5–8.0	3.2-D1 in spread; 2.4-D1 in butter	Margarine intervention-6 wks; butter intervention-5 wks	↔ Serum retinol ↓ α - and β -carotene concentrations	↔ Serum vitamin D ↔ lipid standardized α -tocopherol
[181]	D	Spread	Adults	Moderately hypercholesterolemic		8	↔ retinol ↓ α - and β -carotenes	↔ vitamin D ↔ ratio of α -tocopherol to cholesterol
[62]	D1, D2	Spread	Adults	5.4–7.5	2.3-D1; 2.2-D2	8	↓ β -carotene ↔ β -carotene ↔ α -carotene with or without lipid adjustment	
[19]	C2	Spread	Adults, aged 19–58	<7.5	0.9, 1.6 and 3.3	3.6	↓ α and β carotene and lycopene concentrations reduced ↔ lipid-adjusted carotene levels	↓ α -tocopherol concentrations reduced ↔ lipid standardized α tocopherol
[41]	D	Spread	Adults	Hypercholesterolemic	1 g 3x/d (2 different formulations) or 2 g 3x/d	8	↔ vitamin A	↔ vitamin D
[20]	C2, C3	Spread	Adults aged 18–62	<8	0.8	9	↓ lycopene ↔ α - and β -carotene	
[76]	C, D	Spread	Adults, mean age 49	4.8–7.0	2.0	4	↔ retinol ↔ α carotene or lycopene concentrations, with or without lipid adjustment. ↓ β -carotene decreased after each intervention, but not when lipid adjusted	↔ vitamin D concentration with or without lipid adjustment.
[96]	D	Spread, Shortenin g	Adults aged 18–65	<6.5	2.5 g/d-lunch or in 3 doses of 0.4-breakfast, 0.8-lunch and 1.3-dinner	4	↔ retinol ↔ α -carotene ↓ lycopene and β -carotene after the 3x/d regime ↓ β -carotene by 1x/d regime ↔ in any of the lipid-adjusted concentrations	↓ α -tocopherol after both the 1x and 3x/d regime 1x/d regime, ↔ in lipid-adjusted concentrations
[137]	D	Spread	Children, aged 6	Healthy	1.5	12	↓ β -carotene with and without lipid standardization	↓ α -tocopherol but ↔ in lipid-adjusted concentration
[134]	D	Spread	24 children aged 3–13; 4 parents; 16 healthy family members	Familial hypercholesterolemia	2.2	12	↔ retinol ↓ α and β -carotenes with and without lipid-adjustment (measurements done only in children)	

Table 3: Summary of scientific evidence for the impact of phytosterols on carotenoid status and fat soluble vitamins (Continued)

[39]	C	Spread	Adults 25–64	>5.8	1.5 or 3.0	24	↔ retinol, α -carotene or β -carotene	
[170]	C	Spread, Salad Dressing	Healthy adults		3.0, 6.0 or 9.0	8	↓ α and trans β -carotene reduced in the 9.0 g/day group, but all carotenoid values remained within the normal range	All fat soluble vitamins remained within normal range after treatments
[38]	C	Spread	Adults, aged 21–75	3.4–5.0	1.1 or 2.2	5	↔ in serum retinol, zeaxanthin or cryptoxanthin ↓ trans β -carotene, α -carotene, lycopene and lutein all decreased ↓ lipid-adjusted trans β -carotene	↔ α - or γ -tocopherol, 25 hydroxyvitamin D or phyloquinone
[40]	C	Yogurt-drink	Adults, aged 33–69	Modertally hypercholesterolemic	I	4	↔ vitamin A	↔ vitamin E, ↑ vitamin D (probably as a result of increased skin synthesis of vitamin D due to the time of year)
[70]	C	Yogurt	Adults	Normocholesterolemic	3	4	↓ β -carotene	↑ lipid standardized tocopherol
[79]	C	Spread	Healthy adults		1.8	3	↔ vitamin A ↓ β -carotene	↔ vitamin E
[173]	C, D	Spread	Adults	Hypercholesterolemic	1.9-C 1.8-D	3	↔	↔
[81]	C		Adults aged 48	Normocholesterolemic and hypercholesterolemic	1.6	52	↓ lipid-adjusted α - and β -carotene	↔ lipid-adjusted fat soluble vitamin concentration
[71]	A2, C2	Vegetable oil, partly filled milk	Men aged 29	<5.2	2.2	I	↓ β -carotene bioavailability 57% with C2, 48% with A2; ↓ TRL-retinyl palmitate bioavailability 48% with C2, 32% with A2. No standardization to TAG because TRL-TAG pharmacokinetics were equivalent in all groups	↓ α -tocopherol bioavailability 27% with C2, no effect with A2
[42]	A2	Vegetable oil, partly filled milk	Adults, aged 60	7.0	1.2 or 1.6	4 wk/period	↓ α - and β -carotene, lycopene, and lutein with 1.6 g dose only. ↔ lipid-adjusted α - and β -carotene, lycopene, β -cryptoxanthin, zeaxanthin with either dose. ↓ lipid-adjusted % change in lutein with 1.6 g	↓ α -tocopherol with both doses ↔ lipid-adjusted α -tocopherol with both doses

See Table 1 for abbreviations. TAG, triacylglycerol; TRL, triacylglycerol-rich lipoprotein.

During plant sterol consumption, increasing the consumption of fruits and vegetables to be > five servings and including one or more carotenoid rich source would be enough to avoid reduction in carotenoid levels resulted from plant sterol intake [106].

Conclusions

Based on the positive results from studies examining the effects of low doses of free plant sterols and sterol esters, there is a good likelihood that a minimum dose of 0.8–1.0 g of free sterol and free sterol equivalents will reduce LDL cholesterol by 5% or more, and that this reduction in

LDL cholesterol will correlate with an approximate 6–10% reduction in coronary heart disease risk at age 70 [35,36]. There is also a good likelihood that the reduction in LDL cholesterol within this same dosage could be higher if full compliance of the plant sterol dosage were assured. In studies where subjects were monitored to ensure full compliance, efficacy with a 1.5 to 2 g/d dose ranged from 12–16%. For maximum efficacy of free plant sterols, the plant sterols must be administered in a soluble or microcrystalline form. Efforts must be taken to assure that the free plants sterols remain in this bio-efficacious form during the shelf life of the product. Our dosage recommendation of 0.8–1.0 g of free sterol and free sterol equivalents compares favorably with the FDA interim final rule 21 CFR 101.83 recommending 0.65 g of sterol esters per serving, twice per day in spreads, which is equivalent to 0.8 g of free sterol equivalents per day. As of January 2003, the FDA recognized that the scientific literature supports expanding the health claim to include free forms of plant sterols and stanols, and to include a wider range of products, including low-fat products. The FDA further stated that the science (as of January 2003) shows that the lowest effective daily intake of free phytosterols is 800 mg/d <http://vm.cfsan.fda.gov/~dms/ds-ltr30.html>.

In addition to their cholesterol lowering properties, plant sterols have other promising effects, including anti-cancer, anti-inflammation, anti-atherogenicity, and anti-oxidation activities. Despite the fact that plant sterols reduce the carotenoid levels in adults, it seems that an intake of plant sterols between 0.8–1.0 g is essential to prevent chronic diseases in adult population. The carotenoid lowering effect of plant sterols can be corrected by increasing intake of food that is rich in carotenoids. However, more studies are needed in pregnant and lactating women as well as on children in order to verify the dose required to decrease blood cholesterol without affecting fat-soluble vitamins and carotenoid status.

Plant sterols are naturally occurring molecules that humanity has evolved with, which partially counter the absorption of dietary cholesterol and have other important biological functions described above. The myriad of factors that can affect the efficacy of plant sterols have been explored. The resonating conclusion is that properly solubilized 4-desmethyl plant sterols, in ester or free form, in reasonable doses (0.8–1.0 g of equivalents per day) and in various vehicles including natural sources, and as part of a healthy diet and lifestyle, are important dietary components for maintaining good heart health. Consumption from natural sources should be encouraged for all persons, and consumption of plant sterols in enriched sources should be encouraged following consultation with a clinician. The clinician should be assured that the individual is a responder to plant sterols, and achieves a

reduction in LDL cholesterol. Along these lines, it would be fruitful to monitor individuals for markers of cholesterol absorption before recommending consumption of enriched plant sterols. Those persons who are poor absorbers of dietary cholesterol (on the basis of low levels of serum cholestanol and plant sterols) [175] may not be ideal candidates for consuming plant sterols to lower plasma LDL cholesterol, but may still benefit from plant sterols' other positive effects.

List of abbreviations

ABC, ATP-binding cassette; apo, apolipoprotein; LDL, Low density lipoprotein; PLD, phospholipase D; PUFA, polyunsaturated fatty acids.

Authors' contributions

AB completed the first draft of the article and tables. PJHJ and SSH then contributed substantially to the text and tables, expanding the initial concept, and elaborating and updating specific themes. AB updated the final text. All authors improved overall flow.

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to plant sterols and plant stanols and maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140), and maintenance of normal prostate size and normal urination (ID 714, 1467, 1635) pursuant to Article 13(1) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies was asked to provide a scientific opinion on a list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006. This opinion addresses the scientific substantiation of health claims in relation to plant sterols and plant stanols and maintenance of normal blood cholesterol concentrations, and maintenance of normal prostate size and normal urination. The scientific substantiation is based on the information provided by the Member States in the consolidated list of Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The food constituent that is the subjects of the health claims is plant sterols and plant stanols. The Panel considers that plant sterols and plant stanols are sufficiently characterised.

1 On request from the European Commission, Question No EFSA-Q-2008-1337, EFSA-Q-2008-1500, EFSA-Q-2008-2203, EFSA-Q-2008-2370, EFSA-Q-2008-3642, EFSA-Q-2008-3872, adopted on 11 February 2010, Question No EFSA-Q-2008-1336, EFSA-Q-2008-1354, EFSA-Q-2008-1972, EFSA-Q-2008-1973, EFSA-Q-2008-2717, adopted on 30 April 2010 and Question No EFSA-Q-2008-1501, EFSA-Q-2008-2204, EFSA-Q-2008-2371, adopted on 10 September 2010.

2 Panel members: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. Correspondence: nda@efsa.europa.eu

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Maintenance of normal blood cholesterol concentrations

The claimed effects are “cholesterol”, “cholesterol levels”, “cholesterol metabolism”, “heart health and artery health because of LDL cholesterol maintenance”, “cardiovascular system”, “cholesterol metabolism”, “effet sur le taux de cholestérol sanguin”, “heart health” and “helps to keep normal cholesterol level”. The target population is assumed to be adults. In the context of the proposed wordings, the Panel notes that the claimed effects refer to the maintenance of normal blood cholesterol concentrations. The Panel considers that maintenance of normal blood cholesterol concentrations is a beneficial physiological effect.

The Panel concludes that a cause and effect relationship has been established between the consumption of plant sterols and plant stanols and the reduction of blood cholesterol concentrations.

The Panel considers that in order to bear the claim, a food should provide at least 0.8 g per day of plant sterols/stanols in one or more servings. These amounts can be reasonably achieved in the context of a balanced diet. The target population is adults. The considerations regarding the food matrix expressed by the Panel in a previous opinion in relation to the blood LDL-cholesterol lowering effect of plant sterols and stanols also apply to the present opinion.

With respect to the specified conditions of use, it is suggested that the labelling provisions outlined in Commission Regulation (EC) No 608/2004 shall continue to apply for products making the proposed claim.

Food products containing plant sterols and/or plant stanols may not be nutritionally appropriate for pregnant and breastfeeding women, and for children under the age of five years.

Maintenance of normal prostate size and normal urination

The claimed effects are “prostate health” and “kidney and prostate health”. The Panel assumes that the target population is adult males. In the context of the proposed wordings, the references submitted and the clarifications provided by Member States, the Panel assumes that the claimed effects refer to the maintenance of a normal prostate size and normal urination. The Panel considers that maintenance of normal prostate size and normal urination is a beneficial physiological effect.

In weighing the evidence, the Panel took into account that the only intervention study using pure beta-sitosterol from which conclusions could be drawn found no effect on prostate size, peak urinary flow rate (Q_{max}) or post-void residual urine volume (PVR).

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of plant sterols and plant stanols and maintenance of normal prostate size and normal urination.

KEY WORDS

Plant sterols, plant stanols, blood cholesterol concentrations, prostate size, urination, health claims.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

See Appendix A

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

See Appendix A

EFSA DISCLAIMER

See Appendix B

INFORMATION AS PROVIDED IN THE CONSOLIDATED LIST

The consolidated list of health claims pursuant to Article 13 of Regulation (EC) No 1924/2006⁴ submitted by Member States contains main entry claims with corresponding conditions of use and literature for similar health claims. EFSA has screened all health claims contained in the original consolidated list of Article 13 health claims which was received by EFSA in 2008 using six criteria established by the NDA Panel to identify claims for which EFSA considered sufficient information had been provided for evaluation and those for which more information or clarification was needed before evaluation could be carried out⁵. The clarifications which were received by EFSA through the screening process have been included in the consolidated list. This additional information will serve as clarification to the originally provided information. The information provided in the consolidated list for the health claims which are the subject of this opinion is tabulated in Appendix C.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claims is plant sterols and plant stanols.

In the context of this opinion, the term plant sterols (present as free sterols or esterified) refers specifically to plant sterols from natural sources with a composition as specified in the Commission Decisions authorising the placing on the market of food products with added plant sterols under Regulation (EC) No 258/97⁶. The term “plant stanol ester” refers to a blend of the plant stanols sitostanol and campestanol, which are obtained from the reduction of plant sterols from food grade plant oils (mainly soybean oil) or tall oil or blends thereof.

The Panel notes that claims ID 1234 and 1235 refer to polyphenols present or extracted from Maritime Pine (*Pinus pinaster* Aiton). However, the only reference cited in the list referring to procyanidins (a type of polyphenol) from French maritime pine bark was not accessible to the Panel after having made every reasonable effort to retrieve it (Assouad and Piriou, 2007), and no references on the effects of polyphenols present or extracted from Maritime Pine on blood lipids or any other health outcome were provided.

The Panel considers that the food constituent, plant sterols and plant stanols, that is the subject of the health claims, is sufficiently characterised.

2. Relevance of the claimed effect to human health

2.1. Maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140)

The claimed effects are “cholesterol”, “cholesterol levels”, “cholesterol metabolism”, “heart health and artery health because of LDL cholesterol maintenance”, “cardiovascular system”, “cholesterol metabolism”, “effet sur le taux de cholestérol sanguin”, “heart health” and “helps to keep normal cholesterol level”. The Panel assumes that the target population is adults.

⁴ Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

⁵ Briefing document for stakeholders on the evaluation of Article 13.1, 13.5 and 14 health claims: <http://www.efsa.europa.eu/en/ndameetings/docs/nda100601-ax01.pdf>

⁶ Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43, 14.2.1997, p. 1–6.

In the context of the proposed wordings, the Panel notes that the claimed effects refer to the maintenance of normal blood cholesterol concentrations.

Low-density lipoproteins (LDL) carry cholesterol from the liver to peripheral tissues, including the arteries. Elevated LDL-cholesterol, by convention >160 mg/dL (>4,14 mmol/L), may compromise the normal structure and function of the arteries. High-density lipoproteins (HDL) act as cholesterol scavengers and are involved in the reverse transport of cholesterol in the body (from peripheral tissues back to the liver).

The Panel considers that maintenance of normal blood cholesterol concentrations is a beneficial physiological effect.

2.2. Maintenance of normal prostate size and normal urination (ID 714, 1467, 1635)

The claimed effects are “prostate health” and “kidney and prostate health”. The Panel assumes that the target population is adult males.

In the context of the proposed wordings, the references submitted and the clarifications provided by Member States, the Panel assumes that the claimed effects refer to the maintenance of a normal prostate size and normal urination.

An increase in size of the prostate (i.e. benign prostatic hyperplasia) is common in middle-aged and elderly men and may lead to abnormal storage and voiding of urine, which is characterised by a decrease in the peak urinary flow rate and by an increase in the residual urinary volume. Prostate size and urinary flow as well as storage (increase in urinary frequency, urgency, incontinence and nocturia) and voiding (weak urinary stream, hesitancy, intermittency, straining to void and dribbling) symptoms can be measured by established methods.

The Panel considers that maintenance of normal prostate size and normal urination is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

3.1. Maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140)

In the context of the procedure for the authorisation of health claims, EFSA has issued two opinions on applications for plant sterols (EFSA, 2008a) and plant stanol esters (EFSA, 2008b) pursuant to Article 14 of Regulation (EC) No 1924/2006. EFSA has also issued a general opinion regarding the conditions of use for health claims under Article 14 of Regulation (EC) No 1924/2006 in relation to the consumption of plant sterols and stanols and the reduction of LDL-cholesterol concentrations as a risk factor for coronary heart disease (EFSA, 2009).

The NDA Panel concluded that a clinically significant LDL-cholesterol lowering effect of between 7 % and 10.5 % could be expected by a daily intake of 1.5 - 2.4 g of plant sterols/plant stanols in an appropriate food matrix (e.g. margarine-type spreads, mayonnaise, salad dressings, and dairy products such as milk, yoghurts and cheese) (EFSA, 2009). The Panel also considered that the source of the sterols (vegetable or tall oil), the actual ratio between the most abundant sitosterol and campesterol and the source of fatty acids (butter or vegetable oil) do not have a relevant impact on the size of the blood LDL-cholesterol lowering effect (EFSA, 2008a, b), and that the efficacy in lowering LDL-cholesterol is similar for plant sterols and stanols in the intake range of 1.5 - 2.4 g per day (Katan et al., 2003; Demonty et al., 2009; EFSA, 2009).

In the most recent meta-analysis on the LDL-cholesterol lowering effects of plant sterols/stanols, 84 clinical trials were included (Demonty et al., 2009). In nine of the studies, daily doses of 0.80-1.0 g had been used. In seven of these studies a statistically significant reduction of LDL-cholesterol concentrations (range -0.19 to -0.33 mmol/L) was found (Beer et al., 2001; Hendriks et al., 1999; Hironaka et al., 2006; Niittynen et al., 2007; Sierksma et al., 1999; Ishizaki T, 2003; Vanhanen, 1994). In one study (Matsuoka et al., 2004) no effect was found with free sterols, and in the study by Miettinen and Vanhanen (1994) the reduction in LDL-cholesterol of 0.26 mmol/L was not statistically significant. Plant sterols were used in seven studies, stanols in one study and in another study a mixture of sterols and stanols was tested. The results of these studies indicate statistically significant lowering of LDL-cholesterol concentrations by consuming moderate doses (0.8-1.0 g per day) of plant sterols or stanols in subjects with normal or mildly elevated LDL-cholesterol concentrations. All but one (Hironaka et al., 2006) of the studies mentioned above were conducted with plant sterols or stanols added to foods such as margarine-type spreads, mayonnaise, and dairy products such as milk and yoghurts including low-fat yoghurts (Demonty et al., 2009; EFSA, 2009).

The Panel concludes that a cause and effect relationship has been established between the consumption of plant sterols and plant stanols and reduction of blood cholesterol concentrations.

3.2. Maintenance of normal prostate size and normal urination (ID 714, 1467, 1635)

The references provided included narrative reviews, *in vitro* and animal studies on the mechanisms by which phytochemicals (including plant sterols) could protect against prostate cancer, case control and prospective cohort studies in humans on the relationship between the intake of various phytochemicals (including plant sterols) and the incidence of prostate cancer, and narrative reviews on the role of dietary factors other than plant sterols on prostate cancer risk. The Panel considers that no conclusions can be drawn from these references for the scientific substantiation of the claim.

Two meta-analyses of randomised, placebo-controlled trials (Wilt et al., 1999, 2000) and two randomised, placebo-controlled trials (Berges et al., 1995; Klippel et al., 1997) on the effects of beta-sitosterols on prostate size, urinary flow and lower urinary tract symptoms (LUTS) in subjects with benign prostatic hyperplasia (BPH) were provided, together with a publication reporting on the follow-up of one of the studies (Berges et al., 2000). Both randomised controlled trials (Berges et al., 1995; Klippel et al., 1997) have been considered in the meta-analyses, and both meta-analyses are by the same authors and report on the same randomised controlled trials (Wilt et al., 1999, 2000).

In the meta-analyses by Wilt et al. (1999, 2000), four double-blinded randomised controlled trials (RCTs) including 519 men with BPH were identified and met the inclusion criteria (Berges et al., 1995; Fischer et al., 1993; Kadow and Abrams, 1986; Klippel et al., 1997). Three of the studies used non-glucosidic beta-sitosterol mixtures (beta-sitosterol-beta-D-glucoside <5 %) from different plant extracts at concentrations of 50 % (Berges et al., 1995) and ≥ 70 % (Fischer et al., 1993; Klippel et al., 1997) and daily doses of 60 to 195 mg per day of beta-sitosterol. The Panel notes that beta-sitosterol has been proposed as the active constituent of certain plant preparations which have been investigated in humans with respect to their effects on LUTS in BPH, and that a number of mechanisms by which beta-sitosterol could exert the claimed effect in BPH tissues have been investigated *in vitro*. However, only a small amount of beta-sitosterol is absorbed (<5 %) and no evidence of a plausible mechanism by which it could exert a systemic effect in BPH has been provided. The Panel also notes that the exact composition of the plant preparations used in these studies has not been provided, and therefore the potential contribution of food constituents other than beta-sitosterol to the claimed effect cannot be evaluated. The Panel considers that no conclusions can be drawn from these studies (Berges et al., 1995; Fischer et al., 1993; Klippel et al., 1997) or the meta-analyses (Wilt et al., 1999, 2000) for the scientific substantiation of the claimed effect in relation to plant sterols or beta-sitosterol.

The RCT by Kadow and Abrams (1986) was conducted in 62 males (mean age 67 years, age range 53-81 years) with symptomatic BPH using pure beta-sitosterol-beta-D-glucoside at a dose of 0.30 mg per day as intervention for 24 weeks. Nine subjects dropped out after randomisation. No significant differences between groups were observed with respect to prostate size, peak urinary flow rate (Qmax) or post-void residual urine volume (PVR). Lower urinary tract symptom scores were not assessed.

No evidence of a biologically plausible mechanism by which plant sterols and plant stanols could exert the claimed effect has been provided.

In weighing the evidence, the Panel took into account that the only intervention study using pure beta-sitosterol from which conclusions could be drawn found no effect on prostate size, peak urinary flow rate (Qmax) or post-void residual urine volume (PVR) .

The Panel concludes that a cause and effect relationship has not been established between the consumption of plant sterols and plant stanols and maintenance of normal prostate size and normal urination.

4. Panel's comments on the proposed wording

4.1. Maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140)

The Panel considers that the following wording reflects the scientific evidence: "Plant sterols/stanols contribute to the maintenance of normal blood cholesterol levels".

5. Conditions and possible restrictions of use

5.1. Maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140)

The Panel considers that in order to bear the claim, a food should provide at least 0.8 g per day of plant sterols/stanols in one or more servings. These amounts can be reasonably achieved in the context of a balanced diet. The target population is adults. The considerations regarding the food matrix expressed by the Panel in a previous opinion (EFSA, 2009) in relation to the blood LDL-cholesterol lowering effect of plant sterols and stanols also apply to the present opinion.

With respect to the specified conditions of use, it is suggested that the labelling provisions outlined in Commission Regulation (EC) No 608/2004⁷ shall continue to apply for products making the proposed claim.

Food products containing plant sterols and/or plant stanols may not be nutritionally appropriate for pregnant and breastfeeding women, and for children under the age of five years.

⁷ Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning the labelling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters. OJ L 97, 1.4.2004, p. 44–45.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, plant sterols and plant stanols, which is the subject of the health claims, is sufficiently characterised.

Maintenance of normal blood cholesterol concentrations (ID 549, 550, 567, 713, 1234, 1235, 1466, 1634, 1984, 2909, 3140)

- The claimed effects are “cholesterol”, “cholesterol levels”, “cholesterol metabolism”, “heart health and artery health because of LDL cholesterol maintenance”, “cardiovascular system”, “cholesterol metabolism”, “effet sur le taux de cholestérol sanguine”, “heart health” and “helps to keep normal cholesterol level”. The target population is assumed to be adults. Maintenance of normal blood cholesterol concentrations is a beneficial physiological effect.
- A cause and effect relationship has been established between the consumption of plant sterols and plant stanols and reduction of blood cholesterol concentrations.
- The following wording reflects the scientific evidence: “Plant sterols/stanols help to maintain normal blood cholesterol levels”.
- In order to bear the claim, a food should provide at least 0.8 g per day of plant sterols/stanols in one or more servings. These amounts can be reasonably achieved in the context of a balanced diet. The target population is adults. The considerations regarding the food matrix expressed by the Panel in a previous opinion in relation to the blood LDL-cholesterol lowering effect of plant sterols and stanols also apply to the present opinion. With respect to the specified conditions of use, it is suggested that the labelling provisions outlined in Commission Regulation (EC) No 608/2004 shall continue to apply for products making the proposed claim.
- Food products containing plant sterols and/or plant stanols may not be nutritionally appropriate for pregnant and breastfeeding women, and for children under the age of five years.

Maintenance of normal prostate size and normal urination (ID 714, 1467, 1635)

- The claimed effects are “prostate health” and “kidney and prostate health”. The target population is assumed to be adult males. In the context of the proposed wordings, the references submitted and the clarifications provided by Member States, the Panel assumes that the claimed effects refer to the maintenance of a normal prostate size and normal urination. Maintenance of normal prostate size and normal urination is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of plant sterols and plant stanols and maintenance of normal prostate size and normal urination.

DOCUMENTATION PROVIDED TO EFSA

Health claims pursuant to Article 13 of Regulation (EC) No 1924/2006 (No: EFSA-Q-2008-1336, EFSA-Q-2008-1337, EFSA-Q-2008-1354, EFSA-Q-2008-1500, EFSA-Q-2008-1501, EFSA-Q-2008-1972, EFSA-Q-2008-1973, EFSA-Q-2008-2203, EFSA-Q-2008-2204, EFSA-Q-2008-2370, EFSA-Q-2008-2371, EFSA-Q-2008-2717, EFSA-Q-2008-3642, EFSA-Q-2008-3872). The scientific substantiation is based on the information provided by the Member States in the consolidated list of

Article 13 health claims and references that EFSA has received from Member States or directly from stakeholders.

The full list of supporting references as provided to EFSA is available on: <http://www.efsa.europa.eu/panels/nda/claims/article13.htm>.

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APPENDICES

APPENDIX A

BACKGROUND AND TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Regulation (EC) No 1924/2006 on nutrition and health claims made on foods⁸ (hereinafter "the Regulation") entered into force on 19th January 2007.

Article 13 of the Regulation foresees that the Commission shall adopt a Community list of permitted health claims other than those referring to the reduction of disease risk and to children's development and health. This Community list shall be adopted through the Regulatory Committee procedure and following consultation of the European Food Safety Authority (EFSA).

Health claims are defined as "any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health".

In accordance with Article 13 (1) health claims other than those referring to the reduction of disease risk and to children's development and health are health claims describing or referring to:

- a) the role of a nutrient or other substance in growth, development and the functions of the body; or
- b) psychological and behavioural functions; or
- c) without prejudice to Directive 96/8/EC, slimming or weight-control or a reduction in the sense of hunger or an increase in the sense of satiety or to the reduction of the available energy from the diet.

To be included in the Community list of permitted health claims, the claims shall be:

- (i) based on generally accepted scientific evidence; and
- (ii) well understood by the average consumer.

Member States provided the Commission with lists of claims as referred to in Article 13 (1) by 31 January 2008 accompanied by the conditions applying to them and by references to the relevant scientific justification. These lists have been consolidated into the list which forms the basis for the EFSA consultation in accordance with Article 13 (3).

ISSUES THAT NEED TO BE CONSIDERED

IMPORTANCE AND PERTINENCE OF THE FOOD⁹

Foods are commonly involved in many different functions¹⁰ of the body, and for one single food many health claims may therefore be scientifically true. Therefore, the relative importance of food e.g. nutrients in relation to other nutrients for the expressed beneficial effect should be considered: for functions affected by a large number of dietary factors it should be considered whether a reference to a single food is scientifically pertinent.

⁸ OJ L12, 18/01/2007

⁹ The term 'food' when used in this Terms of Reference refers to a food constituent, the food or the food category.

¹⁰ The term 'function' when used in this Terms of Reference refers to health claims in Article 13(1)(a), (b) and (c).

It should also be considered if the information on the characteristics of the food contains aspects pertinent to the beneficial effect.

SUBSTANTIATION OF CLAIMS BY GENERALLY ACCEPTABLE SCIENTIFIC EVIDENCE

Scientific substantiation is the main aspect to be taken into account to authorise health claims. Claims should be scientifically substantiated by taking into account the totality of the available scientific data, and by weighing the evidence, and shall demonstrate the extent to which:

- (a) the claimed effect of the food is beneficial for human health,
- (b) a cause and effect relationship is established between consumption of the food and the claimed effect in humans (such as: the strength, consistency, specificity, dose-response, and biological plausibility of the relationship),
- (c) the quantity of the food and pattern of consumption required to obtain the claimed effect could reasonably be achieved as part of a balanced diet,
- (d) the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.

EFSA has mentioned in its scientific and technical guidance for the preparation and presentation of the application for authorisation of health claims consistent criteria for the potential sources of scientific data. Such sources may not be available for all health claims. Nevertheless it will be relevant and important that EFSA comments on the availability and quality of such data in order to allow the regulator to judge and make a risk management decision about the acceptability of health claims included in the submitted list.

The scientific evidence about the role of a food on a nutritional or physiological function is not enough to justify the claim. The beneficial effect of the dietary intake has also to be demonstrated. Moreover, the beneficial effect should be significant i.e. satisfactorily demonstrate to beneficially affect identified functions in the body in a way which is relevant to health. Although an appreciation of the beneficial effect in relation to the nutritional status of the European population may be of interest, the presence or absence of the actual need for a nutrient or other substance with nutritional or physiological effect for that population should not, however, condition such considerations.

Different types of effects can be claimed. Claims referring to the maintenance of a function may be distinct from claims referring to the improvement of a function. EFSA may wish to comment whether such different claims comply with the criteria laid down in the Regulation.

WORDING OF HEALTH CLAIMS

Scientific substantiation of health claims is the main aspect on which EFSA's opinion is requested. However, the wording of health claims should also be commented by EFSA in its opinion.

There is potentially a plethora of expressions that may be used to convey the relationship between the food and the function. This may be due to commercial practices, consumer perception and linguistic or cultural differences across the EU. Nevertheless, the wording used to make health claims should be truthful, clear, reliable and useful to the consumer in choosing a healthy diet.

In addition to fulfilling the general principles and conditions of the Regulation laid down in Article 3 and 5, Article 13(1)(a) stipulates that health claims shall describe or refer to "the role of a nutrient or other substance in growth, development and the functions of the body". Therefore, the requirement to

describe or refer to the 'role' of a nutrient or substance in growth, development and the functions of the body should be carefully considered.

The specificity of the wording is very important. Health claims such as "Substance X supports the function of the joints" may not sufficiently do so, whereas a claim such as "Substance X helps maintain the flexibility of the joints" would. In the first example of a claim it is unclear which of the various functions of the joints is described or referred to contrary to the latter example which specifies this by using the word "flexibility".

The clarity of the wording is very important. The guiding principle should be that the description or reference to the role of the nutrient or other substance shall be clear and unambiguous and therefore be specified to the extent possible i.e. descriptive words/ terms which can have multiple meanings should be avoided. To this end, wordings like "strengthens your natural defences" or "contain antioxidants" should be considered as well as "may" or "might" as opposed to words like "contributes", "aids" or "helps".

In addition, for functions affected by a large number of dietary factors it should be considered whether wordings such as "indispensable", "necessary", "essential" and "important" reflects the strength of the scientific evidence.

Similar alternative wordings as mentioned above are used for claims relating to different relationships between the various foods and health. It is not the intention of the regulator to adopt a detailed and rigid list of claims where all possible wordings for the different claims are approved. Therefore, it is not required that EFSA comments on each individual wording for each claim unless the wording is strictly pertinent to a specific claim. It would be appreciated though that EFSA may consider and comment generally on such elements relating to wording to ensure the compliance with the criteria laid down in the Regulation.

In doing so the explanation provided for in recital 16 of the Regulation on the notion of the average consumer should be recalled. In addition, such assessment should take into account the particular perspective and/or knowledge in the target group of the claim, if such is indicated or implied.

TERMS OF REFERENCE

HEALTH CLAIMS OTHER THAN THOSE REFERRING TO THE REDUCTION OF DISEASE RISK AND TO CHILDREN'S DEVELOPMENT AND HEALTH

EFSA should in particular consider, and provide advice on the following aspects:

- Whether adequate information is provided on the characteristics of the food pertinent to the beneficial effect.
- Whether the beneficial effect of the food on the function is substantiated by generally accepted scientific evidence by taking into account the totality of the available scientific data, and by weighing the evidence. In this context EFSA is invited to comment on the nature and quality of the totality of the evidence provided according to consistent criteria.
- The specific importance of the food for the claimed effect. For functions affected by a large number of dietary factors whether a reference to a single food is scientifically pertinent.

In addition, EFSA should consider the claimed effect on the function, and provide advice on the extent to which:

- the claimed effect of the food in the identified function is beneficial.
- a cause and effect relationship has been established between consumption of the food and the claimed effect in humans and whether the magnitude of the effect is related to the quantity consumed.
- where appropriate, the effect on the function is significant in relation to the quantity of the food proposed to be consumed and if this quantity could reasonably be consumed as part of a balanced diet.
- the specific study group(s) in which the evidence was obtained is representative of the target population for which the claim is intended.
- the wordings used to express the claimed effect reflect the scientific evidence and complies with the criteria laid down in the Regulation.

When considering these elements EFSA should also provide advice, when appropriate:

- on the appropriate application of Article 10 (2) (c) and (d) in the Regulation, which provides for additional labelling requirements addressed to persons who should avoid using the food; and/or warnings for products that are likely to present a health risk if consumed to excess.

APPENDIX B

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of the food/food constituent, a positive assessment of its safety, nor a decision on whether the food/food constituent is, or is not, classified as foodstuffs. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wordings of the claims and the conditions of use as proposed in the Consolidated List may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 13(3) of Regulation (EC) No 1924/2006.

APPENDIX C

Table 1. Main entry health claims related to plant sterol/plant stanols, including conditions of use from similar claims, as proposed in the Consolidated List.

ID	Food or Food constituent	Health Relationship	Proposed wording
549	Plant Sterols	Heart Health <u>Clarification provided</u> Plant Sterols improve blood cholesterol levels Daily Phytosterols intake helps achieve acceptable LDL- cholesterol levels	Shown to reduce levels of cholesterol by reducing its absorption into the blood.
		Conditions of use - 800mg/day	
ID	Food or Food constituent	Health Relationship	Proposed wording
550	Plant sterols	Cholesterol levels	Plant sterols may help support healthy blood cholesterol levels
		Conditions of use - 2 g/day - Taimsete steroolide sisaldus tootes 2 g/100 g, maksimaalselt võib päevas tarbida 3 g taimseid stereoole - 200-300mg is the daily minimum dose recommended from dietary sources for optimum health. The most recent study used 1.3g. A meta-analysis of 41 trails - No RDA / RNI - 20 – 100 milligram (mg)	
ID	Food or Food constituent	Health Relationship	Proposed wording
567	Plant stanol ester	Cardiovascular system <u>Clarification provided</u> For cholesterol management Inhibits/blocks the absorption of dietary cholesterol	Contains plant stanols that effectively reduce cholesterol. Reduces effectively cholesterol levels Actively reduces cholesterol. Proven to reduce cholesterol. Clinically proven to reduce cholesterol. Lowers cholesterol. Reduces blood cholesterol. Lowers blood cholesterol. Reduces LDL (bad) cholesterol. For cholesterol management. Symbol included in the claim: Benecol (see previous)

<p>Conditions of use</p> <ul style="list-style-type: none"> - 2 g/day - Consume 2g of plant stanol, provided as plant stanol ester foods, per day preferably with a meal. Consumption at the recommended intake. Mandatory labelling statements required as per Commission Regulation EC No 608/2004 - Consume 2g of plant stanol, provided as plant stanol ester, per day preferably with a meal. Consumption at the recommended intake. Mandatory labelling statements required as per Commission Regulation EC No 608/2004: Intended exclusively for people who want to lower their cholesterol level; patients on cholesterol lowering medication should only consume the product under medical supervision; products may not be nutritionally appropriate for pregnant and breast feeding women and children under the age of 5 years; the product is to be used as part of a balanced and varied diet, including regular consumption of fruit and vegetables to help maintain carotenoid levels; consumption of more than 3g/d should be avoided). 			
<p>Comments from Member States</p> <p>FI comments: Cholesterol reduction claims are considered to be in the scope of Art. 14. Claim Ref.nr 60849 is not a cholesterol lowering claim and that's why this claim must be addressed separately. Other subclaims under ID 567 do not support the subclaim Ref.nr 60849 and should therefore be addressed separately. (Ref.nr 52212 phytosterols/sterols claim: heart healths, different conditons of use, different substance; Ref.nr 63259 sterols/stanols and their esters claim: heart health, different substance). Under the claim ID 561 there is a subclaim Ref.nr 60848. It does not belong under ID 561 but should be under ID 567.</p>			
ID	Food or Food constituent	Health Relationship	Proposed wording
713	Phytosterols (mixture of Beta-sitosterol, Campesterol, Stigmasterol, Brassicasterol, Stigmastanol, Ergostanol, Campestanol)	Cholesterol metabolism	Contributes to normal cholesterol level in blood
<p>Conditions of use</p> <ul style="list-style-type: none"> - Min. 1 g per day 			
ID	Food or Food constituent	Health Relationship	Proposed wording
714	Phytosterols (mixture of Beta-sitosterol, Campesterol, Stigmasterol, Brassicasterol, Stigmastanol, Ergostanol, Campestanol)	<p>Prostate health</p> <p><u>Clarification provided</u></p> <p>Phytosterols contribute to the normal functioning of the prostate:</p> <p>Help to reduce oxidative damage of prostate cells and tissue</p> <p>Help to keep your prostate in shape</p>	Contributes to normal functioning of prostate and urinary tract
<p>Conditions of use</p> <ul style="list-style-type: none"> - 280 mg/day 			

ID	Food or Food constituent	Health Relationship	Proposed wording
1234	Barre céréalière diététique contenant des stérols végétaux et des polyphénols de pin maritime(OPC)	effet sur le taux de cholestérol sanguin,	anti-oxydant, Les stérols végétaux sont reconnus pour maîtriser l'excès de cholestérol. Les polyphénols extrait de l'écorce de pin permettent la réduction des lipides oxydés à la surface des artères
	Conditions of use - 750 g de stérols et 30 mg de polyphénol par portion, 3 portions maximum par jour soit 2, 25 g de stérol et 90 mg de polyphénol (OPC) produit ciblé adulte présentant un taux élevé de cholestérol		
	No clarification provided by Member States		
ID	Food or Food constituent	Health Relationship	Proposed wording
1235	Stérols et polyphénols (Complément alimentaire sous forme de comprimé)	effet sur le taux de cholestérol sanguin,	anti-oxydant, Les stérols végétaux sont reconnus pour maîtriser l'excès de cholestérol. Les polyphénols extrait de l'écorce de pin permettent la réduction des lipides oxydés à la surface des artères
	Conditions of use - 350 mg de stérols et 30 mg de polyphénols (OPC) par comprimé. 4 comprimés par jour à prendre de façon régulière		
	No clarification provided by Member States		
ID	Food or Food constituent	Health Relationship	Proposed wording
1466	Beta sitosterol	Cholesterol	functions by displacing cholesterol from intestinal micelles, thus reducing cholesterol absorption
	Conditions of use - 1 g/day - Bakery products with $\geq 6g/100g$ of wheat grain fibre		
ID	Food or Food constituent	Health Relationship	Proposed wording
1467	Beta sitosterol	Kidney and prostate health <u>Clarification provided</u> Kidney and prostate health: Nucleotides modulate the immune response by enhancing the production of immunoglobulins and improve T-cell function Nucleotides are immunostimulating agents	Helps maintain normal kidney and prostate function

		Beta sitosterol contributes to the normal functioning of the prostate	
Conditions of use <ul style="list-style-type: none"> - Amount of consumption: 60 mg/Tag - Min 60 mg per day 			
ID	Food or Food constituent	Health Relationship	Proposed wording
1634	Phytosterols (mixture of Beta-sitosterol, Campesterol, Stigmasterol, Brassicasterol, Stigmastanol, Ergostanol, Campestanol)	Cholesterol metabolism	Contributes to normal cholesterol level in blood
Conditions of use <ul style="list-style-type: none"> - minimum of 800 mg phytosterols/stanols - Min. 1 g per day - At least 700 mg per day 			
ID	Food or Food constituent	Health Relationship	Proposed wording
1635	Phytosterols (mixture of Beta-sitosterol, Campesterol, Stigmasterol, Brassicasterol, Stigmastanol, Ergostanol, Campestanol)	Prostate health <u>Clarification provided</u> Prostate health. Phytosterols contribute to the normal functioning of the prostate. Help to reduce oxidative damage of prostate cells and tissue.	Contributes to normal functioning of urinary tract
Conditions of use <ul style="list-style-type: none"> - 280 mg/day - 100 mg tägl Nahrungsergänzung– 			
ID	Food or Food constituent	Health Relationship	Proposed wording
1984	Phytostanols / sterols	heart health <u>Clarification provided</u> Cholesterol metabolism: contributes to normal cholesterol level in blood (Health relationship and example claims altered to be in line with claim 1634 which has no comment from EFSA.)	Plant sterols/ stanols help to maintain a healthy heart
Conditions of use			

	<ul style="list-style-type: none"> - At least 800mg stanols/sterols per daily dose - only with a minimum of 800 mg phytosterols / stanols /day 		
ID	Food or Food constituent	Health Relationship	Proposed wording
2909	Sterols/ stanols and their esters	Heart health and artery health because of LDL cholesterol maintenance	Sterols/ stanols and their esters promote heart health/keep your arteries healthy/l
	<p>Conditions of use</p> <ul style="list-style-type: none"> - Consume at least 2g plant sterols/stanols provided as plant sterol ester, per day. Consumption at the recommended intake for optimal effect. Mandatory labelling statements required as per Commission Regulation EC no 608/2004; Intended exclusively for people who want to lower their cholesterol level; patients on cholesterol lowering medication should only consume the product under medical supervision; products may not be nutritionally appropriate for pregnant and breast feeding women under the age of 5 years; the product is to be used as part of a balanced diet, including regular consumption of fruit and vegetables; consumption of more than 3 g/d is not efficacious 		
ID	Food or Food constituent	Health Relationship	Proposed wording
3140	betasitosterol	helps to keep normal cholesterol level	helps to keep normal cholesterol level, helps to keep passage of vessels, natural way to avoid risks caused by high cholesterol values
	<p>Conditions of use</p> <ul style="list-style-type: none"> - 1080 mg of betasitosterol per day 		

GLOSSARY AND ABBREVIATIONS

HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LUTS	Lower urinary tract symptoms
BPH	Benign prostatic hyperplasia
RCT	Randomised controlled trial
Qmax	Peak urinary flow rate
PVR	Post-void residual urine volume