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Accumulation of phytosterols in food

Evaluation of the adverse effects following the intake of high dose of phytosterols

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RAPPORT IN HET KORT

Accumulatie van fytosterolen in de voeding

Op de markt verschijnen steeds meer producten, die verrijkt zijn met plantensterolen (fytosterolen). De gecombineerde consumptie van dergelijke producten kan leiden tot overdosering, zodat er mogelijk aanvullende maatregelen nodig zijn om overdosering te voorkomen.

Fytosterol-verrijkte voeding verlaagt het plasma LDL-cholesterol. Een dagelijkse inname van 1-3 g plantensterolen verlaagt de LDL-cholesterol concentratie met 5-15%; een hogere inname geeft geen extra effect. Door hun slechte absorptie geven fytosterolen geen systemische toxiciteit. Fytosterolen verlagen echter wél de absorptie van bèta-carotenoïden, die van belang zijn voor de aanmaak van vitamine A. De consumptie van (margarine verrijkt met) 3 g fytosterol per dag gedurende een jaar leidt tot een 33% afname van de bèta-caroteen spiegel, die echter (alleen) zorgelijk is bij risicogroepen met een hoge vitamine A behoefte, zoals zwangeren, moeders die borstvoeding geven en jonge kinderen. Er is overigens geen indicatie voor fytosterolgebruik door deze groepen, maar het gebruik kan niet worden uitgesloten.

De beschikbare data over schadelijke effecten bieden geen basis voor het stellen van een maximaal toelaatbare dagelijkse doses voor fytosterolen. Evenals de Gezondheidsraad en SCF, wordt thans aanbevolen om niet meer (meer dan 3 g per dag) van deze plantensterolen in te nemen, omdat a. hogere doseringen niet effectiever zijn, en b. langetermijnstudies ontbreken. Op termijn zijn beleidsmaatregelen nodig ter voorkoming van overdosering in gebruikers- en risicogroepen.

Trefwoorden: fytosterol, plantensterol, LDL-cholesterol, accumulatie, voedingssupplement

ABSTRACT

Accumulation of phytosterols in food

Phytosterol-enriched foods (sterol or stanol esters) decrease plasma LDL-cholesterol in healthy and hypercholesterolaemic individuals; a daily intake of 1-3 g plant sterols lowers LDL-cholesterol levels by about 5-15%. A higher intake of phytosterols is not more effective.
Phytosterols are poorly absorbed and retain no systemic toxicological effects i.e. no endocrine, teratogenic, mutagenic or carcinogenic effects. However, plant sterols and stanols interfere with the absorption of bèta-carotenoids, but have virtually failed to appear in plasma levels of other fat-soluble vitamins, such as vitamin A, D, E and tocopherols. One-year consumption of margarine enriched with phytosterol (3 g per day) decreased plasma bèta-carotenoid by 33%. However, the consequences of such a persistent decrease of blood concentrations of bèta-carotene on human health are largely unknown. Bèta-carotene as vitamin A precursor has not been deduced as giving serious cause for concern except in cases where the vitamin A requirement is higher than normal, as in pregnancy, lactation or infancy. Note that phytosterols are not indicated for these groups, but their use can not be excluded.
The available data on adverse effects provide no basis for setting an upper limit to total daily intake of phytosterols, but considering, first, that the observed maximum effective dose for lowering cholesterol is 2-3 g/day and, second, that long-term studies are lacking, it would seem prudent to avoid the intake of plant sterol in amounts exceeding this dose. This advise corresponds with the advice given by the Dutch Health Council and SCF. Since a number of foods appear as potential 'candidates' for enrichment with plant sterols, additional regulatory measures may be needed to avoid excessive intakes.

Key words: phytosterol, plant sterols, LDL-cholesterol, accumulation, food supplement

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LIST OF ABBREVIATIONS

HDL:	high density lipoprotein
LDL:	low density lipoprotein
Tc:	total cholesterol
LDLc:	LDL-cholsterol

SAMENVATTING

Fytosterolverrijkte voeding verlaagt het plasma LDL-cholesterol in gezonde individuen en hypercholesterolemie-patiënten; een dagelijkse inname van 1-3 g plantsterolen verlaagt de LDL-cholesterol met 5-15%. Een hogere inname geeft geen extra effect.

Fytosterolen worden slecht geabsorbeerd, bezitten geen systemische toxiciteit en vertonen geen endocriene, teratogene, mutagene of carcinogene effecten. Plantsterolen en -stanolen beïnvloeden echter wél de absorptie van bèta-carotenoïden; de plasma-concentraties van andere vet-oplosbare vitamines, zoals vitamine A, D, E en tocoferolen worden ogenschijnlijk niet beïnvloed. De consumptie (van margarine verrijkt met) 3 g fytosterol per dag gedurende een jaar leidt tot een 33% afname van de bèta-caroteen spiegel en dit nadelige effect dient meegenomen te worden bij de risicoschatting van met fytosterolen verrijkte voedingsmiddelen, zeker bij langetermijngebruik. De consequentie van zo'n persistente daling in de plasma bèta-caroteen concentratie op de gezondheid is niet bekend. Vitamine A wordt uit bèta-caroteen aangemaakt, zodat deze daling (alleen) zorgelijk is als de vitamine A behoefte groter is dan normaal, zoals in de zwangerschap, bij het geven van borstvoeding en bij consumptie door zeer jonge kinderen. Er is overigens geen indicatie voor fytosterolgebruik door deze groepen, maar het gebruik ervan kan niet worden uitgesloten.

De beschikbare data over negatieve effecten bieden geen basis voor het stellen van een maximaal toelaatbare dagelijkse dosis voor fytosterolen. Evenals de Gezondheidsraad en SCF, wordt thans aanbevolen om niet meer (dan 2-3 g per dag) van deze plantensterolen in te nemen, omdat a. hogere doseringen niet effectiever zijn, en b. lange termijn studies ontbreken. Indien de consumptie van fytosterolen beduidend toeneemt tengevolge van een hoger aanbod van fytosterolbevattende produkten, zijn beleidsmaatregelen nodig ter voorkoming van overdosering in de gebruikers- en genoemde risicogroepen.

SUMMARY

Phytosterol-enriched foods (sterol or stanol esters) decrease plasma LDL-cholesterol in healthy and hypercholesterolaemic individuals; a daily intake of 1-3 g plant sterols lowers LDL-cholesterol levels by about 5-15%. Higher intake of phytosterols is not more effective.
Phytosterols are poorly absorbed and retain no systemic toxicological effects i.e. no endocrine, teratogenic, mutagenic or carcinogenic effects. However, plant sterols and stanols interfere with the absorption of bèta-carotenoids, but virtually not the plasma levels of other fat-soluble vitamins, such as vitamin A, D, E and tocopherols. One-year consumption of margarine enriched with phytosterol (3 g per day) decreased plasma bèta-carotenoid by 33%. The consequences of such a persistent decrease of blood concentrations of bèta-carotene on human health are largely unknown. No serious concern can be deduced regarding the role of bèta-carotene as a vitamin A precursor, except in cases where the vitamin A requirement is larger than normal, like in pregnancy, lactation or infancy. Note that phytosterols are not indicated for these groups, but their use can not be excluded.
The available data on adverse effects do not provide a basis for setting an upper level of total daily intake of phytosterols but considering the observed maximal effective dose for cholesterol lowering is 2-3 g/day and the lack of long-term studies, it is prudent to avoid plant sterol intakes exceeding this dose. This advise corresponds with the advice given by the Dutch Health Council and SCF. Since a number of foods appear as potential candidates to be enriched with plant sterols, additional management measures may be needed to avoid excessive intakes.

1. INTRODUCTION

Phytosterols have a long history of use. Bèta-sitosterol has been used since the 1950s as a supplement and a drug, marketed in the U.S. and Canada under the name of Cytellin and Positol, respectively for the treatment of hypercholesterolemia [1]. Due to poor solubility, this drug prescribed at doses as high as 25 g per day, consumed in solid crystalline form, had limited efficacy. It is estimated that 2,400 subjects have taken part in clinical studies with phytosterol and stanols with dosages up to 25 g or more per day. Cytellin was prescribed for more than 20 years and had an excellent safety record. Since 1990s, phytosterols have been introduced in fatty foods.

Sterols are widely present in plant foods; cooking oils and margarines (containing between 100 and 500 mg per 100 g) are the main sources of plant sterols in typical Western diets. Legumes (up to 220 mg/100g) and some seeds (e.g. sunflower and sesame 500 to 700 mg per 100 g) are also good sources, while other vegetables and fruits contain slightly lower amounts of plant sterols. Most of us eat 150-400 mg of phytosterols daily, which is almost equivalent to that of cholesterols. Consumption by vegetarians is even higher i.e. 450 mg daily [2]. If phytosterol-enriched food supplements are consumed, the daily intake of phytosterol will be 5 to 10 times higher.

Based on the induced decrease of serum cholesterol by phytosterols, the expected reduction of the risk of cardiovascular disease is estimated to be some 15-40% [3, 4], an effect considered to be more significant than reducing the intake of saturated fat. Based on the benefits of the observed decreases in blood cholesterol, it was claimed that consumption of margarines, enriched with plant sterols or stanols, is expected to reduce the risk of heart disease by 25% [3]. No studies are, however, available confirm a beneficial effect of phytosterol intake on the incidence of cardiovascular disease. The trials available have involved only relatively small numbers of subjects and have been of relatively short duration.

2. PRESENT REGULATORY STATUS

Recently published reports by scientific committees that advise regulatory authorities on phytosterol supplementation in food, like the Scientific Committee on Food [5-8], Australian New Zealand Food Authority [9], and the Committee on Safety Assessment of Novel Foods of the Health Council of the Netherlands (Committee VNV) [10-12]examined adverse effects of the increased intake of phytosterols. These reports, describing data relevant for adverse effects of accumulating doses of phytosterols, have been incorporated in the present review.

One unresolved question is whether absorption of plant sterols, albeit very small, could result in adverse effects over time. Some regulatory bodies have not considered this a significant issue and the products are regarded as safe. In the opinion of the Dutch Health Council, however, a safe upper level of 9.1 g phytosterols daily for an individual with a weight of 70 kg has an insufficient scientific basis. At such intake levels, as well as at lower advised levels, biological effects may arise. In addition, there is as yet insufficient information on the clinical relevance in the long term.

Moreover, the Dutch Health Council and the American Department of Health and the American Heart Association [13] have advised against the use of phytosterol supplements in pregnant women and young children, as they do not need to reduce their blood cholesterol levels and there is a possibility that the products could affect their vitamin A status.

Recent post launch monitoring studies of “yellow fat spreads with added phytosterol esters” indicated no adverse health effects from the current intake of marketed spreads containing phytosterol esters [8, 14]. Finally, the FDA has accepted plant sterol/stanol esters are asserted to be Generally Recognized as Safe (GRAS) by manufacturers.

Comment by the authors:

> *Health and regulatory authorities consider phytosterol enriched foods as relatively safe. Yet, there is insufficient information on the clinical relevance in the long term. Children and pregnant women are at increased risk as they are not indicated to reduce their blood cholesterol and their vitamin A status may be affected by phytosterol supplements.*

3. EFFICACY OF PHYTOSTEROLS

The efficacy of plant sterol preparations to lower blood cholesterol of patients with type II hyperlipoproteinaemia has been evaluated by Lees et al. [15] in a total of 46 patients. As reported by Raulio et al. [16] the study included different groups and doses, including subjects receiving up to 18 g of sterols per day in their diet and the average duration of the test diet was 10 months. Nutrition markers were not measured in this study. There were no outward signs of any side effects. The maximal mean cholesterol-lowering effect in response to any preparation was 12%, although it was much greater in some individual patients. Sterol balance data showed that plant sterols inhibit cholesterol absorption with a maximal negative cholesterol balance in adults at a dose of 3 g/day of a tall oil sterol suspension [16].

Another example is the study of Miettinen et al. [17] who performed a one-year, randomized, double-blind study in 153 randomly selected subjects with mild hypercholesterolemia. Fifty-one subjects consumed margarine without sitostanol ester (the control group), and 102 consumed margarine containing sitostanol ester (1.8 or 2.6 g of sitostanol per day). The reductions in LDL-cholesterol was 14.1% in the sitostanol group and 1.1% in the control group. More recently, it was shown that the improvements of LDLc, HDLc, Tc, apolipoprotein B concentrations, and LDL/HDL cholesterol ratio during the daily consumption of a phytosterol ester-enriched margarine were most marked in subjects with a high dietary intake of cholesterol, energy, total fat and saturated fatty acids, and with a high baseline absorption [18].

Law et al. [3] have reviewed and summarised the results of several randomised, double-blind trials in human adults that compared the ability of foods (thirteen polyunsaturated margarines, five mayonnaises, one olive oil, and one butter) with and without added plant sterols to lower cholesterol. In summary, average daily doses between 0.8 an 4.0 g/day significantly reduced Tc and LDLc levels, with only marginal change in blood HDLc or triglyceride. Higher responses were noted at 2 g/day as compared with 1 g/day, while maximal effects were seen at 2 g/day.

A meta analysis of 41 trials showed that intake of 2 g/day of stanols or sterol reduced LDL-cholesterol by 10% [19]. The response is greater with daily intakes of about 2 g as compared to 1 g, while no further increase was noted at intakes above 2 g [19]. Taking the dose once (2.5 g at lunch) produced the same effect as 2.5 g devided over three meals [20].

Plant sterols enhance the LDL-cholesterol lowering effect of reduced-fat consumption [21]: eating low cholesterol and saturated fat foods and high in sterols reduce LDL-cholestrol by 20%, and adding sterols or stanols to statin medication is more effective than doubling the

statin dose [22].

4. SAFETY OF PHYTOSTEROLS

The safety studies on phytosterol esters as particular novel food components have already been assessed by various scientific committees that advise regulatory authorities on phytosterol supplementation in food, like the Scientific Committee on Food [5-8], the Australian New Zealand Food Authority [9], and the Committee on Safety Assessment of Novel Foods of the Health Council of the Netherlands (Committee VNV) [10-12].

In healthy adult men and women, the safety and tolerability of esterified phytosterols in reduced-fat spreads and/or in salad dressings have been assessed in a study of eight weeks [23]. In a randomised, double-blind, parallel study design, eighty-four subjects consumed reduced-fat spread and salad dressing providing 0, 3, 6 or 9 g of phytosterols per day. The only laboratory abnormalities detected were, at the higher levels of 6-9 g per day, some changes in alanine transferase and creatine kinase activities. Such levels generally experience important fluctuations in response to external factors. Significant reductions were observed in alpha- and bèta-carotene in the group receiving 9 g phytosterols per day.

Hendriks et al. showed in a one-year follow-up study on the use of low fat spread enriched with plant sterol esters [24] no adverse effects after daily consumption of 1.6 g phytosterols. Significant reductions of blood alpha- and bèta-carotene concentrations were, however, observed, whereas the levels of the fat-soluble vitamins A, K_1, D and E were not affected.

Individuals with an inborn error of phytosterol metabolism are at risk when they take phytosterol-enriched foods; worldwide only 50 cases are known. These subjects hyper-absorb and retain not only cholesterol but also all other sterols that are not catabolised but deposited in tissues, including vascular intima.

As mentioned above, the Dutch Health Council and the American Department of Health and the American Heart Association [13] have advised against the use of phytosterol supplements in pregnant women and young children. These subgroups are not indicated to reduce their blood cholesterol levels and phytosterols may affect their vitamin A status due to a decrease in b-carotene blood level.

Tammi et al. [25] performed a double blind crossover intervention study in 81 children at 6 years of age. The study showed that the replacement of 20 g of the child’s dietary fat intake with plant stanol ester margarine for 3 months significantly reduced blood LDLc, but decreased the serum B-carotene to LDLc ratio.

Another point of concern of increased phytosterol intake is the concomitant use of cholesterol-lowering medication. Two studies have shown that treatment with statins increased the phytosterol/cholesterol ratio by increased absorbtion. For example, an increase in the ratio of sitosterol/cholesterol by 200% has been observed [26, 27], implying a potentially dangerous interaction.

Comment by the authors:

Except from significant reductions of blood alpha- and bèta-carotene no adverse health effects have been observed in subjects receiving 1 g phytosterols per day for one year or 9 g phytosterols per day for 8 weeks. Children and pregnant women are at risk as their vitamin A status may be affected by phytosterol supplements. Finally, people with phytosterolaemia and patients receiving cholesterol-lowering medication should consume phytosterol-enriched foods under medical supervision.

4.1. Endocrine and teratogenic effects

Animal studies have shown that plant sterols and stanols are poorly absorbed but mainly detected in the adrenal glands and ovaries. Several animal studies indicated (revised by the Scientific Committee on Food [5]), that plant sterols, especially sitosterol, at high level or when administered subcutaneously retain potential oestrogenic activity [28]. Clear oestrogenic effects were found in fish [28, 29]. In the rat subcutaneous administration of 0.5 to 5 mg/kg per day caused a significant reduction in both sperm count and the weights of testes [30]; application of sitosteryl ester to the vagina of rabbits significantly lowered their pregnancy rates [31].

In a series of in vitro assays with vegetable oil plant sterols, it has been shown that they do not bind to either the human oestrogen receptor or the immature rat oestrogen receptor. Furthermore, uterotrophic assays with immature female rats orally treated with phytosterols (47.9% ß-sitosterol, 28.8% campesterol, 23.3% stigmasterol) and phytosterol esters (47.3% ß-sitosterol, 28.1% campesterol, 24.1% stigmasterol) in doses of 5, 50 and 500 mg/kg bw per day for 3 days did not reveal any oestrogenic response using uterine weights as end point. In addition, the same mixture of phytosterols induced no oestrogenic activity in a recombinant yeast assay, nor did they bind to cytosolic oestrogen receptors in the rat uterine [32]. Plant stanol esters have similarly been proven not to possess any oestrogenic potential in an in vitro assay (proliferation of MCF-7 cells) or in a rat uterotrophic assay in vivo [33].

In the mustelid European polecat [34], an increase in plasma estradiol and thyroid hormones, as well as some alterations in intermediary metabolism, was seen at doses of about 5 mg/kg body weight/day. This suggests that phytosterols have the potency to act as endocrine disruptors. It should be noted that sterols did not decrease blood cholesterol in the polecat, but significantly increased it. In addition, the endocrine effects previously described in fish were different from those observed in the polecat. The observed effects seem therefore not relevant for the situation in humans. Finally note, that the effects of phytosterols observed in the polecat hardly seemed harmful.

Rats receiving a diet of up to 5% total stanols (equivalent to 2.4 - 3.5g stanols/kg bw per day) during a gestation period of 21 days showed no adverse treatment-related maternal or foetal developmental effects i.e. no significant differences in uterine weight, placental weight, foetal weight, number of foetuses, implantation sites or corpora lutea and early and late/late resorptions [35].

In a two-generation reproduction study in rats, phytosterol esters had no effect on the reproduction of the F0 and F1-generations, nor on the development of the F1-and F2-pups, nor on the sexual maturation of the F1-weanlings nor on oestrous cycles. A dietary phytosterol ester concentration of 8.1% (4000 mg/kg/day vegetable oil sterol esters in the diet; equivalent to 5% free plant sterols) was shown to be the no-observed-adverse-effect level (NOAEL) which was equivalent to a dose of 2.5-9.1 g/kg bw per day (depending on the period during the study) [36]. In a second two generation reproductive toxicity study [37], rats were treated with plant sterols in diet at 0, 1, 2.5 and 5%. Like in the study of Waalkens-Berendsen et al., fertility and reproductive parameters were not altered by the treatment in either generation. The calculated NOEL in this study was 4.5%. Based on the two-generation reproductive studies in rats, the Scientific Committee on Food [8] stated that sufficient reassurance was provided of the absence of endocrine effects via the oral route. Finally, in a 3-week study with 12 men and 12 women who consumed 5.8 g phytosterols per day no changes in the sex hormone levels in females was shown [38].

4.2. Mutagenic effects

A major breakdown product of cholesterol is 4-cholesten-3-one. This product and its major faecal by-product 5-bèta-cholestan-3-one showed no mutagenic activity in the bacterial mutation assay and the in-vitro chromosome aberration assay [39]. Neither 4-cholesten-3-one, nor 5-bèta-cholestan-3-one showed mutagenic activity in the Salmonella typhi mutation assay.

Both substances showed no clastogenic potential in the chromosome aberration assay in vitro [40, 41].

Phytosterols (47.9% ß-sitosterol, 28.8% campesterol, 23.3% stigmasterol) and phytosterol-esters (47.3% b-sitosterol, 28.1% campesterol, 24.1% stigmasterol) show neither mutagenic activity in the bacterial mutation assay with Salmonella typhimurium nor clastogenic activity in the chromosomal aberration test (both in the presence and absence of S-9 mix derived from rat livers). In addition, two genotoxicity studies were conducted in vivo using a phytosterol ester mixture containing 0.3% cholesterol, 3.0% brassicasterol, 28.1% campesterol, 0.8% campestanol, 18.7% stigmasterol, 45.5% ß-sitosterol, 2.6% ß-sitostanol, 1.1% D5-avenasterol and 1.9% others. This phytosterol mixture did not induce unscheduled DNA synthesis in the livers of orally dosed male rats (once with 2000 mg/kg). In another study in rats the plant sterol mixture did not induce micronuclei in the polychromatic erythrocytes of bone marrow of male rats treated up to 2000 mg/kg/d [5].

Phytosterol oxides, generated under extreme heating conditions, retains no genotoxicity or obviousevidence of toxicity, as well; a NOEL value for these oxides of 128-144 mg/kg/day [42].

In 2000, the Minister for Health, Welfare and Sport asked the Committee on Safety Assessment of Novel Foods of the Health Council of the Netherlands (Committee VNV) to review all data critically cf. [10]. This committee considered the significance of the exploratory genotoxicity and cytotoxicity research on the metabolite 4-cholesten-3-one described in the scientific literature as low [43-45]. This was based on the following considerations: The nuclear aberration test (NA) is an unvalidated test that has not been officially acknowledged. The parameters determined indicate a general toxic effect rather than a specific genotoxic effect. This finding can probably be induced with very many substances. The sister chromatid exchange test (SCE) is also not yet internationally accepted. The mechanism is unclear, and the biological relevance is debatable. In addition, 4-cholesten-3-one gives only a marginally positive response. The Committee VNV further considers the genotoxicity tests conducted in accordance with the international protocol to be decisive and regards the phytosterols and cholesterol metabolites tested as non-genotoxic.

5. EFFECTS ON VITAMINS

5.1. Reduction in blood levels of carotenoids and other nutrients

The main concern related to the consumption of foods enriched in sterol esters is the reduction in the absorption of fat-soluble vitamins, especially bèta-carotene. A 20% reduction in serum bèta-carotene level is estimated to occur following one year ingestion of 20 g per day of products containing 8% free phytosterols [5]. Such a reduction in blood bèta-carotene levels seems relevant for subjects with a non-optimal vitamin A status, although the bèta-carotene concentration remained within the normal range and within normal seasonal variation. In addition, some other potential benefits of carotenoids not directly related to vitamin A formation can also become compromised [5].

In addition to bèta-carotene, phytosterols also decrease, though smaller i.e. 5-15%, the blood level of α-carotene, lycopene, vitamin E, and α-tocopherol. Mensink et al. [46] recently showed that plant stanol esters (3 g per day) decreased the blood level of several carotenes (α-carotene, bèta-carotene, lycopene) and xantophyles (lutein/zeaxanthin and bèta-cryptoxanthin). The expected reduction in absolute blood tocopherol concentrations was not observed here. Actually, the LDL-cholesterol particles were enriched in tocopherols. After standardisation for LDLc, levels of the various tocopherols were significantly increased, those of various carotenoids were unchanged and those of bèta-carotene were decreased. This may suggest that changes in antioxidant concentrations cannot be simply explained by a decrease in the number of circulating LDL-cholesterol particles [46].

A meta analysis of 10 to 15 studies per vitamin showed that the plasma concentrations of alpha-carotene, bèta-carotenen and lycopene were significantly reduced by 9%, 28% and 7%, respectively, by stanols or sterols at 1.5 g/day or more. In contrast, the plasma levels of vitamin A and D were not affected [19]. After correction for the reduction in LDL-cholesterol, only the decline in bèta-carotene level remained significant. It has been calculated that decreases in blood carotenoids plateaued when doses of sterols or stanols reached 2.2 g per day [47].

In a study of one year’s consumption of sitostanol ester margarine [48], the decrease versus controls of bèta-carotene was 33.3% and that of α-carotene 19.5%; the decreases versus the baseline home diet were 25% and 10%, respectively. The decrease remained significant when the decrease in bèta-carotene was corrected for the decrease in cholesterol. No significant effects were observed for vitamin D and retinol concentrations, or for the ratios of α-tocopherol to cholesterol and of α-carotene to cholesterol.

A subsequent long-term (52 weeks) follow-up study on the use of a plant sterols enriched spread [49] showed a similar decrease in carotenoids. However, as compared with the study of Gylling et al. [48] only a 6% reduction in LDLc was observed. Similar results were obtained in other long-term study on safety and efficacy of phytosterol esters in 185 volunteers following controlled intake of 20 g per day of a spread-containing phytosterol esters (equivalent to 1.6 g sterols/day) over a one-year period [17, 24] i.e. a beneficial reduction in the blood Tc and LDLc levels and a reduction in the absorption of the most lypophilic carotenoids (e.g. bèta-carotene).

5.2. Effects of long-term intake of carotenoids

In assessing long-term studies, one should be aware that plasma levels of carotenoids can vary seasonally by up to 30% depending on the availability of fruit and vegetables [50, 51], so that variation in the carotenoid level do not raise concerns *per se*.

The phytosterol-induced decrease of blood bèta-carotene concentration proves to be of no substantial concern because the levels of bèta-carotene have always been found to remain within the “normal” range. Different intervention studies have shown that this range of baseline values for blood bèta-carotene is large (from 0.2 to 1.7 μM). Studying the factors that influence the absorption and distribution of carotenoids and other nutrients in adolescents showed a large variability in blood levels of bèta-carotene (10th percentile, 0.11 μM; 90th percentile, 0.41 μM) and lycopene (10th percentile, 0.25 μM; 90th percentile, 0.78 μM [52]. The relationship of carotenoids levels with any physiological function or health marker remained, however, unclear.

Dietary carotenoids, particularly bèta-carotene, are important precursors of vitamin A. Consumption of carotenoids is even more important in countries (i.e. Asia and Africa), where the intake of animal products is low, because preformed vitamin A is only present in those products (e.g. liver, eggs, milk products. But also in developed countries, carotenoids may usually contribute to vitamin A supply by more than 40%. As such, in countries where vitamin A deficiency is common, or in situations where vitamin A requirements are greater than normal (i.e. pregnancy, lactation, infancy), the phytosterol-induced decrease in blood bèta-carotene may give serious health problems. On the other hand, it appears that in the phytosterol-enriched food trials, blood retinol levels remained unchanged despite the consistently observed decreases in bèta-carotene.

In addition to its vitamin A precursor function, carotenoids have been suggested to serve as radical quencher, antioxidant, anti-carcinogen, and regulator of other cellular functions [6]. Still, there is no convincing evidence that a decrease of up to one third of bèta-carotene levels, or of other nutrients that are lowered by phytosterol over-consumption, affects these functions.
The bèta-carotene reduction was not felt an essential concern in a trial of a sitostanol ester margarine [48] considering two studies on harmful effects of bèta-carotene supplementation [53, 54] and the observation that blood retinol concentration was not affected. The harmful effects were, however, observed in studies using 20-30 mg per day of synthetic all-trans bèta-carotene, not natural sources of bèta-carotene. Such doses largely exceed the usual dietary daily dose. Natural dietary sources may contribute in Europe about 3-7 mg per day of bèta-carotene [7], which is known to retain beneficial health benefits. There may be a very small difference between the levels of bèta-carotene that may produce adverse effects in smokers (20 mg per day in the ATBC study), and those that may confer health benefits to the general population (up to 10 mg per day) mainly from natural sources, according to previous statements of the Committee [6, 7]. As such, it is advocated to increase the intake of natural sources of
bèta-carotene, i.e. carotenoid-rich vegetables and fruits, to counterbalance the expected reduction of the levels of bèta-carotene and of other fat-soluble nutrients during prolonged consumption of phytosterol-enriched foods.
Indeed, Noakes et al. [55] showed in a well-controlled study that, when consuming plant sterol esters (2.3 g per day) or stanol esters (2.5 g per day) in spreads, a moderate increase of dietary carotenoids (an additional daily serving of high-carotenoid vegetables or fruits) may effectively maintain blood carotenoid concentrations. The dietary advice resulted in a 13% increase of
bèta-carotene in subjects who consumed for three weeks the sterol-free control spread. LDLc decreased by 7.7% and 9.5% after consumption of sterol ester- and stanol ester-enriched spreads, respectively, and there were no significant differences in the blood bèta-carotene concentrations (standardised by Tc plus triglycerides) of control, stanol ester and sterol ester groups.
No adverse effects, except of the reduction in carotenoids, were observed following consumption of stanols at doses as high as 3 g per day for three years [3]. Finally note that stanol margarines have been sold for five years in Finland without overt evidence of hazards.

Comment by the authors:

In general, a tendency is apparent from the literature that the longer the duration of

phytosterol consumption the larger the decrease in blood carotenoid concentration by some 20-25% i.e. bèta-carotenoid by about 25%, alpha-carotene by about 10% and vitamin E by about 8%. Enrichment of the products with carotenoids does not prevent this decrease in serum carotenoids. Such decreases must, however, be viewed in perspective. The carotenoid levels remain within the levels of normality and within the degree of variability encountered among individuals, and plasma levels of carotenoids can vary seasonally by up to 30% depending on the availability of fruit and vegetables [50, 51].

6. SUBCHRONIC STUDIES

6.1 Animal data

Rats were fed phytosterols for 90 days at 0 to 8.1% in the diet [56], but no adverse effects were observed (post-mortem examination including heamatology, blood clinical chemistry, and histology). The same findings were observed in a similar study where rats were dosed phytosterols by gavage up to 9 g/kg per day during 13 weeks [57].

Mice were dosed with 2% w/w tall-oil non-esterified plant sterols in diet for 18-weeks [58]. Animal deaths during the study, any adverse clinical signs, or changes in blood glucose were not reported. Canges in haematological parameters (haemoglobin concentration, red cell counts and hematocrit) were not observed between controls and treated groups, except for a significant reduction (20%; $p<0.05$) in mean platelet counts in the treated group compared to controls. Upon gross examination, no abnormalities were observed except for two controls having skin lesions. Histological examination demonstrated focal and non-specific inflammation in the pelvic calyces of the kidneys and hepatic vacuolation in livers of control mice. Arrested spermatogenesis and atrophy in the seminiferous tubules was observed to a variable extent in controls and treated groups. The results showed that plant phytosterols in the diet at up to 2% w/w was well tolerated by mice over a period of 18 weeks and there was no reduction in growth rate. In treated groups, some small non-specific histological changes in the kidney and reductions in platelet counts were observed, but they appeared not to be clinically significant as they were also observed in the controls.

6.2 Human data

In a double-blind placebo-controlled multi-centre study, subjects (N=150, but completed by 113 subjects) with primary hypercholesterolaemia aged 21 to 70 years received 1.8 g phytosterols per day (divided into three doses per day, and supplied in cereal-based nutritional bar) for 8 weeks [59]. Following 8-week treatment at a dose of 1.8 g per day total and LDL cholesterol were reduced by 3.8 and 3.4%, respectively. During the study a total of
55/134 subjects reported one adverse event (31 in the placebo group and 24 in the active treatment period). Reported clinical signs ranged from skin, respiratory, cardiovascular, gastrointestinal and musculo-skeletal effects and psychiatric troubles, but these complaints and symptoms were not related to gender, treatment, and given dose. Clinical chemistry parameters

were not significantly different in placebo and treatment groups.

In another double blind placebo controlled study, 132 subjects received phytosterols at doses of 0.9, 1.8 or 3.6 g per day in milk-based drinks for 28 days [60]. In all treatment groups (including the placebo group) mild to moderate adverse clinical effects were reported in 52% of subjects (ranging from general symptoms, skin, respiratory, cardiovascular, gastrointestinal and musculoskeletal effects). These clinical signs and symptoms were, however, not related to gender, to treatment, to the phytosterol dose. Though, overall there were significant increases in weight among all subjects, the differences were independent on treatment. Except for an increase in systolic blood pressure at a dose of 0.9 g per day compared to placebo, no significant increases in blood pressure or pulse rate post treatment were observed in the different groups. At the end of the treatment, subjects who were had received a daily dose of 0.9 g showed significantly increased platelet counts and eosinophils. At a two-fold higher dose significant increases were noted in red blood cell counts, haemoglobin and haematocrit, and alkaline phosphatase during treatment. In the placebo group post-treatment, alanine transaminase was increased and uric acid decreased. However, none of the changes in blood chemistry differed between the four treatment groups. Subjects on whom urinalysis were performed was small and as such no statistical tests of significance other than specific gravity and pH were performed.

Vitamin A and E and alpha and bèta-carotene levels were measured both at the start of the study and at 4 weeks post-treatment. Except in subjects dosed at 1.8 g per day (significant 11% lower mean vitamin A levels as compared to placebo, there were no differences between treatment groups at the start of treatment. At week 4 post-treatment vitamin A was significantly reduced: 10% ($p<0.005$), 12% ($p<0.001$) and 9% ($p<0.01$) as compared to placebo following treatment with 0.9, 1.8 and 3.6 g per day, respectively. There was, however, no dose-dependency regarding the decrease in vitamin A. Except for a significant ($p<0.01$) 23% reduction in mean alpha-carotene level in subjects dosed at 3.6 g per day, no significant differences between groups with respect to vitamin E, alpha or bèta-carotene levels were observed.

Using the same study design, the effect of margarines enriched with sterols from soybean, sheanut or ricebran oil or with sitostanol-ester at a dose of 1.5 to 3.3 g per day was evaluated with non-enriched margarine as control during four periods of 3.5 weeks [4]. Two thirds of the soybean oil sterols were esterified to fatty acids. The study was performed in 100
(95 volunteers completed the study) healthy non-obese normocholesterolaemic and mildly hypercholesterolaemic volunteers (aged 45 ± 12.8 y) with plasma total cholesterol levels below

8 mM at entry. Compared to control, margarines enriched in soybean oil sterol-esters or sitostanol-ester significantly reduced plasma total- and LDL-cholesterol (LDLc) concentrations by 8-13% with no effect on HDL-cholesterol level. The LDL- to HDL-cholesterol ratio was reduced by 0.37 and 0.33 units for these margarines, respectively. All sterol enriched margarines reduced lipid-standardised plasma alpha- plus bèta-carotene levels. Plasma lycopene levels were also reduced but this effect was not significant for all products. None of the margarines adversely affected blood clinical and haematological parameters, or serum total bile acids.

The same laboratory (Unilever Research Laboratories) recently performed another randomised double-blind placebo-controlled parallel trial on 185 healthy volunteers (35-64 y). Subjects daily consumed for one year 20 g spread enriched with 1.6 g plant sterols as fatty acid esters or a control spread [24]. Plant sterol ester-enriched spread significantly lowered total and LDLc during the one year period on average by 4 and 6%, respectively. Plant sterols intake did on average not result in a lower carotenoid concentration (when expressed per LDLc) after 52 weeks ($P>0.05$) though carotenoid concentrations changed over time. After 1 year of plant sterols intake lipid adjusted alpha- and bèta-carotene-concentrations was reduced by 15-25% as compared to control spread, whereas lipid-adjusted fat-soluble vitamin concentrations remained unchanged. Serum campesterol and bèta-sitosterol significantly increased ($P<0.0001$) from 2.76 to 5.31 (µmol/mmol total cholesterol, and from 1.86 to 2.47 (µmol/mmol total cholesterol), respectively. The increase in total plant sterol concentration in red blood cells did not affect red blood cell deformability. Free and total testosterone levels in males, luteinising hormone, follicle stimulating hormone, bèta-estradiol and progesterone in females, as well as various clinical chemical and haematological parameters measured were not affected by the treatment. Adverse events reported were not different between subjects consuming control spread and subjects consuming plant sterol esters-enriched spread.

7. CHRONIC STUDIES

7.1. Animal data

A diet with 5% ß-sitosterols from tall oil and from cottonseed oil were fed to groups of 10 rats each for periods of 8, 18 and 22 months, respectively. No effects were detected with respect to growth, blood cell counts, blood urea nitrogen, serum proteins and gross or microscopic appearance of any organ or tissue [61]. In the same study [61], 11 dogs were fed a diet with ß-sitosterols at doses of 0.5 and 1 g per kg body weight for 8 and 22 months. ß-Sitosterol diet did not significantly affect body weight, haematological parameters, serum composition and the results of gross and microscopic pathological examination as compared to two control dogs. Similarly, New Zealand rabbits of both sexes were fed for 50 to 120 weeks with 3% cottonseed oil and 4% ß-sitosterols derived from either tall oil or cottonseed oil. Total liver and aorta lipid concentration and free and total liver and aorta sterol concentrations were essentially the same as those observed in two control rabbits, and no gross or microscopic abnormality of the blood vessels or other tissues were observed after consuming 4 g of ß-sitosterols per day [61].

7.2. Human data

Long-term clinical studies (> 4 years) with continuous administration of ß-sitosterols to patients (unfortunately the number and dose were not given) did not show any harmful effect with respect to kidney and liver function, blood and urine composition, electrocardiogram, and gall bladder visualisation. No symptoms indicating a contribution of ß-sitosterols to vascular lesions [61] were observed. Finally, Finland has some six years experience from the use of stanol-enriched fat spreads on the market. No harmful effects have been reported, though it should be mentioned that data were not actively collected. No other long-term studies in humans (other than have already been mentioned in the previous sections) have been published.

8. GASTROINTESTINAL ABSORPTION OF STEROLS

Sitostanol is very poorly absorbed in humans [62] and animals [63]. Studies to gastrointestinal absorption of sterols by humans showed that compared with the percent absorption for cholesterol of 56.2 ± 12.1% obtained in normal subjects under the same test conditions the absorption was 0.04% for sitostanol and 0.15-0.19% for campestanol [62, 64].

In another study, with an intake of 105 mg sitosterol/day, five subjects absorbed 7.5 ± 2.2% of sitosterol by plasma turnover, whereas another absorbed 5% with the use of the radioactive dual-isotope ratio method [65]. In a previous study, a single subject had sitosterol absorption of 4% by the dual radioactive isotope procedure while consuming 300 mg sitosterol per day [66]. Finally, three subjects consuming 145 mg of sitosterol/day had sitosterol absorption of 5 ± 1% in a well validated faecal recovery study [62]. In summary, the absolute amount of sitosterol absorbed is very small, even when the material is made bioavailable by emulsification with lecithin. In adition, absorption of phytosterols does not linearly increase with the dose, indicating that the absorption of plant sterols is a saturable process.

9. CONCLUSIONS

The efficacy of phytosterol-enriched foods to diminish hypercholesterolaemia (reduction of blood LDLc) has been clearly demonstrated. A daily intake in the range of 1-3 g plant sterols lowers LDLc levels by about 5-15%, in different populations, ages and conditions. Maximal effects are observed at a dose of 1-3 g per day. Though it may be anticipated, there is no hard evidence that phytosterol intake lowers the incidence of cardiovascular disease.

A potential serious side effect of phytosterol intake is the interference with the absorption of fat-soluble vitamins, notably bèta-carotenoids. The plasma levels of other fat-soluble vitamins, such as vitamin A, D, E and tocopherols are virtualy not affected. The decreases in blood carotenoids appear to plateau when doses of sterols or stanols reach 2.2 g per day and amount to a reduction of 33% after one-year consumption of an enriched margarine providing 3 g per day. This adverse effect has to be considered for an appropriate risk assessment of the consumption of phytosterol-enriched products, particularly in a long-term perspective.

The health consequences of such a persistent decrease in blood bèta-carotene are largely unknown. As bèta-carotene is a precursor of vitamin A, there is serious concern in situations where vitamin A requirements are greater than normal as in pregnancy, lactation or infancy. In addition, it is advised to consume more carotenoid-rich vegetables and fruits, to counterbalance the expected reduction of blood bèta-carotene and other fat-soluble nutrients levels.

Plant sterols are much less well absorbed than cholesterols. Still, short-term studies show that their consumption gives a small but dose-dependent increase in plasma phytosterol. Individuals with an autosomal recessive disease i.e. sitosterolaemia are, however, at increased risk, because very high plasma levels of phytosterols lead to severe and premature atherosclerosis. The available studies provide no evidence that low levels of phytosterols induce adverse effects, but information on the adverse effects of long-term exposure to higher levels of plant sterols is lacking.

The available data on adverse effects give no basis for a justified numerical upper level of total daily intake of phytosterols. As doses beyond 2 g/day give no additional effect and the safety of higher intakes has not been established, it is advocated to limit the daily intake to a dose of 3 g. Additional management measures may be needed to avoid excessive intakes, considering the increase on the market of food products that contain phytosterols.

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