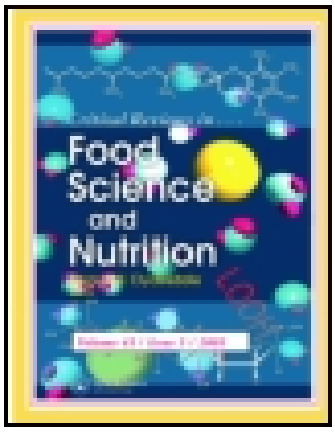

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Influence of Phytosterol and Phytostanol Food Supplementation on Plasma Liposoluble Vitamins and Provitamin A Carotenoid Levels in Humans: An Updated Review of the Evidence

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Influence of phytosterol and phytostanol food supplementation on plasma liposoluble vitamins and provitamin A carotenoid levels in humans: An updated review of the evidence

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Abstract

Phytosterols and phytostanols (PAP) compete with cholesterol absorption in the intestine, resulting in a 5-15%-reduction in plasma total and LDL cholesterol. An important issue is the PAP potential to reduce the plasma concentrations of fat-soluble vitamins and provitamin A carotenoids. Here, an update of the scientific evidence is reviewed to evaluate plant PAP-enriched foods impact on plasma fat-soluble vitamins and carotenoid levels, and to discuss potential implications in terms of cardiovascular risk. Based on 49 human interventional and 3 bioavailability studies, results showed that regular consumption, particularly over the long term, of foods fortified with PAP as recommended in labelling does not significantly impact plasma vitamins A, D and K concentration. A 10% significant median reduction was observed for □-tocopherol. Concerning carotenoids, while 13 studies did not demonstrate statistically significant plasma β-carotene reduction, 20 studies showed significant reductions, with median effect size of -24%. This decline can be mitigated or offset by increased fruits and vegetables consumption. Furthermore, higher cardiovascular risk was observed for differences in plasma □-carotene concentration of the same magnitude as the estimated average decrease by PAP consumption. These results are supported by the only study of β-carotene bioavailability showing decrease in absorption by phytosterols daily intake.

INTRODUCTION

Phytosterols and phytostanols (PAP) are natural compounds found in plant products, notably in grains, plant oils (e.g., pine tree oil for industry), and some fruits and vegetables. They have a similar structure to cholesterol but differ in their side chain at C24 and/or the position and configuration of the double bonds. Phytostanols are produced by hydrogenating phytosterols. These compounds compete with cholesterol absorption in the intestine, resulting in LDL cholesterol reduction that ranges from 5-15% (EFSA Panel on Dietetic Products, 2012; Ras et al., 2013), a high level of LDL cholesterol being a well-known cardiovascular risk factor (Kritchevsky and Chen, 2005). Phytosterols and phytostanols have been incorporated into various food vectors, mainly in margarines, accompanied by health claims.

The most recent review of the literature agrees on the average dose-response effect of PAP on LDL cholesterol (Musa-Veloso et al., 2011). The meta-analysis by Ras *et al*. reported that intake of 0.3-3.2 g of plant sterols reduces LDL-cholesterol by 8.5% (Ras et al., 2013). The ANSES (French Agency for Food, Environmental and Occupational Health & Safety) concluded that intakes of 1.5-2.4 g/d PAP reduce total and LDL-cholesterol by approximately 10 % over both short and medium durations (1-2 years) (ANSES, 2014). At similar daily levels of intake, PAP exhibit similar effects (ANSES, 2014).

An important issue regarding the effectiveness and nutritional value of PAP is their potential to reduce plasma concentrations of fat-soluble vitamins, including tocopherols (vitamin E) and carotenoids, some being precursors of vitamin A (*e.g.*, □-carotene as provitamin A) (Rocha et al., 2011).

Therefore, the effect of PAP on fat-soluble vitamin absorption has been thoroughly studied. In 2003, a meta-analysis of 18 studies concluded that PAP significantly decreased the serum concentration of α-carotene and β-carotene by 8.7% and 19.9%, respectively. After adjusting those reductions to the reduction in plasma cholesterol, the decrease was significant for β-carotene only (estimated at -12.1%) (Katan et al., 2003). However, the meta-analysis procedure was not reported in this paper, and several studies (Amundsen et al., 2002; Hallikainen et al., 1999; Judd et al., 2002; Nestel et al., 2001; Plat et al., 2000; Relas et al., 2001; Tammi et al., 2000; Volpe et al., 2001; Weststrate et al., 1998) were not included in the analysis, without clear rationale.

Furthermore, a reduction in the level of circulating carotenoids is considered a potentially important concern because low plasma carotenoid levels have been inversely associated with the risk of several chronic diseases (Eliassen et al., 2012; Goyal et al., 2013; Ito et al., 2006b; Ribaya-Mereado and Blumberg, 2004), notably coronary artery diseases (Kritchevsky, 1999; Voutilainen et al., 2006). Here, we propose an updated review of the scientific evidence to investigate the impact of PAP-enriched foods on plasma fat-soluble vitamins and carotenoid levels and discuss the potential implications in terms of cardiovascular risk.

FAT-SOLUBLE COMPOUNDS AND PHYSIOLOGICAL MECHANISMS INVOLVED

The fat-soluble vitamins are vitamins A, D, E (including four tocopherols and four tocotrienols) and K. Natural vitamin A exists in two main forms: 1) The active form of vitamin A (also called

retinol) found in animal products (e.g., retinyl esters) such as fish oil and liver; this form can be used directly by the body; and 2) vitamin A precursors (*i.e.*, provitamin A) found in plants as α-, β- and □-carotene and β-cryptoxanthin. Vitamin A activity results from the conversion of these carotenoids, among which β-carotene is the greatest contributor (Yonekura and Nagao, 2007) because it is the most abundant in food and exhibits the most efficient conversion. Vitamin D is the generic name for compounds exhibiting the biological activity of cholecalciferol (Vitamin D3). This compound is the main dietary source of vitamin D and it is present mostly in foods of animal origin. A fraction of vitamin D3 is produced endogenously in the skin from 7-dehydrocholesterol by the action of UV light. Vitamin D2 (ergocalciferol) is a substance synthetized by plants from UV irradiation. Diet can also provide 25-hydroxycholecalciferol and trace amounts of dihydroxycholecalciferol (Borel, 2003). Vitamin E is a generic term that refers to a group of compounds that include both tocopherols and tocotrienols. Four tocopherols (α-, β-, γ- and δ-tocopherols) and four tocotrienols (α-, β-, γ- and δ-tocotrienols) occur naturally, α-tocopherol being the most biologically active form of vitamin E, and d-α and d-γ-tocopherols being the main dietary sources of vitamin E. For example, γ-tocopherol is the most common in the North American diet; and γ-tocopherol can be found in corn oil, soybean oil, margarine, and dressings (Borel, 2003). The term vitamin K is used as a generic descriptor of 2-methyl-1,4-naphthoquinone and all its derivatives that exhibit an anti-hemorrhagic activity in animals fed a vitamin K-deficient diet. They comprise a substance that is synthetized by plant only, phylloquinone (vitamin K1) also known as phytomenadione or phytonadione, and a family of bacterially synthesized menaquinones (vitamin K2) (Borel, 2003).

The metabolism of fat-soluble vitamins and carotenoids in the upper gastrointestinal tract varies depending on the species (Borel, 2003). The precise mechanisms by which phytosterols reduce plasma concentrations of fat-soluble vitamins are the interaction with micellar absorption in the intestine and/or the interaction with carrier lipid particles in the blood (Ntanios and Duchateau, 2002). Thus, phytosterols may interact with vitamin D absorption by competing for incorporation into mixed micelles and by competing for enterocyte capture *via* NPC1L1 (Goncalves et al., 2010). The mechanisms associated with carotenoids have not been well identified beyond the basic understanding that the lipophilic nature of these compounds could explain their mal-absorption in the presence of PAP. It is thought that β-carotene (which is an apolar hydrocarbon compound) is solubilised within mixed micelles (Yonekura and Nagao, 2007 123 398), whereas oxygenated carotenoids such as β-cryptoxanthin are located at the surface of micelles (Borel et al., 1996). Therefore, PAP would replace not only cholesterol in the core of mixed micelles but also other compounds that are present in micelles.

EARLY CONCLUSIONS REGARDING THE ADVERSE EFFECT OF PLANT STEROLS/STANOLS ON FAT-SOLUBLE VITAMINS

The potential impact of PAP on fat-soluble vitamin absorption has been reviewed at the time of the evaluation for their utilization as food ingredient. In 2000, commercialisation approval for these foods was issued with the condition that the labelling state that “the product may not be nutritionally appropriate for certain sections of the population (pregnant and breast-feeding

women and children under the age of five years)" and that "the product should be used as part of a healthy diet, including regular consumption of fruit and vegetables (to help maintain carotenoid levels)" (European Commission - Health & Consumer Protection Directorate-General, 2000a). This warning was required because "a reduction in the level of β-carotene in plasma is to be considered as regards with individuals whose vitamin A status is not optimal, especially pregnant and lactating women and young children. Therefore, it is necessary to inform consumers that the product lowers the level of β-carotene and to give them appropriate dietary advice on the regular consumption of fruits and vegetables" (European Commission - Health & Consumer Protection Directorate-General, 2000a).

Indeed, the Opinion of the Scientific Committee on Food of the European Commission reported in 2000 that "at 11-13% of phytosterols in the fat spreads, no appreciable effect on the fat-soluble vitamins calciferol, tocopherols and phylloquinone was noticed, but a 10% reduction of □- and □-carotene as well as lycopene was observed. This reduction of 10% itself seems not to be of physiological relevance, but, considering a long term exposure and taking into account the 97.5 percentile of intake, the decline of □-carotene levels might be higher" (European Commission - Health & Consumer Protection Directorate-General, 2000b). In 2002, Ntanios and Duchateau concluded that "The carotenoid status could be maintained with an average daily intake of around 100 g vegetables, of which a considerable percentage may not be rich in carotenoids. Further, adopting a healthy diet with the daily intake of plant sterol esters almost fully negates the reduction effect on carotenoids (Noakes et al., 2002)" and that "to date, there is insufficient evidence to conclude that variation in blood carotenoid levels has any established

consequence for either good health or disease development (Lux and Naidoo, 1994)" (page 37) (Ntanios and Duchateau, 2002).

AN UPDATED REVIEW OF THE EVIDENCE

A REVIEW OF HUMAN INTERVENTIONAL STUDIES

Study selection

All controlled intervention studies (with parallel or cross-over designs) investigating the effects of PAP on plasma fat-soluble vitamin and provitamin A carotenoid levels in humans have been collected from PubMed and ISI Web of Knowledge databases up to December 2014. Percent changes in fat-soluble and carotenoid plasma levels have been calculated from the differences in plasma fat-soluble and carotenoid before and after the intervention with PAP-enriched food vectors (as indicated in **Table 1**). They were expressed either based on absolute plasma concentrations or based on standardized values by total or LDL-cholesterol plasma values (see Tables 1 & 2). Among the 52 intervention studies, bioavailability studies (which were usually conducted over shorter periods than usual intervention studies) and studies including foods enriched with fat-soluble vitamins and/or their precursors, and studies including diets enriched with fruits and vegetables (containing fat-soluble vitamins and/or their precursors) were excluded from calculations, that is a total of 14 studies (see **Table 1**). Due to the non-Gaussian

distribution of calculated percentage changes for the remaining 38 intervention studies, average reductions have been expressed as the median (non-parametric statistics) (see **Table 2**).

Study characteristics

Fifty-two intervention studies conducted in humans between 1998 and 2013 have evaluated the effect of supplementation with PAP on plasma or serum levels of fat-soluble vitamins and/or their precursors (**Table 1**). Among these studies, 3 are *sensu stricto* bioavailability studies (Granado-Lorencio et al., 2011; Relas et al., 2001; Richelle et al., 2004), and 1 has been published only in abstract form (Kaffe et al., 2012). The doses of PAP vary widely: in most cases, the selected doses are those known to have an effect on LDL-cholesterol, *i.e.*, 2-3 g/day (median=3 g/day); in other cases, the doses were either <2 g/d (from 1.1 to 1.8 g/d) (Amundsen et al., 2004; Hansel et al., 2007; Hendriks et al., 2003; Sanchez-Muniz et al., 2009; Tuomilehto et al., 2009) or in the 5-9 g/day range (Clifton et al., 2004; Gylling et al., 2010; Mensink et al., 2010; Tuomilehto et al., 2009).

Concerning food vectors, margarine is by far the most widely used vector, followed distantly by dairy products (milk and yogurt types) and cereal products (pastries, breakfast cereals, oat beverage and bread). In one study, PAP were ingested without a vector (Kaffe et al., 2012). The studies were most commonly based on a double-blind parallel design and less frequently (approximately one-third) according to a cross-over design. The number of subjects varied from 15 to 318. The durations of PAP supplementations varied and were most often 3 to 8 weeks (median = 8 weeks) with few long-term studies, with only 5 studies conducted for longer than 5

months) (Amundsen et al., 2004; Christiansen et al., 2001; Gylling et al., 2010; Gylling et al., 1999; Hendriks et al., 2003).

The diet associated with PAP supplementation was generally not modified and was sometimes a low-fat (Hallikainen et al., 1999; Homma et al., 2003) or a moderately low-fat diet (Hernández-Mijares et al., 2010; Judd et al., 2002; Mensink et al., 2002; Sanchez-Muniz et al., 2009); however, in some studies, information regarding diet was not provided. In 3 studies, the effect of an increase in fruit and vegetable intake was studied (Amundsen et al., 2002; Clifton et al., 2004; Noakes et al., 2002). Low-fat diets also have been studied as the basic diet for both control and test groups (Andersson et al., 1999; Hernández-Mijares et al., 2010). Finally, some PAP-enriched test meals were also enriched with fat-soluble vitamins (Amundsen et al., 2004; Brufau et al., 2004; Granado-Lorencio et al., 2011; Hallikainen et al., 2000; Nestel et al., 2001; Quílez et al., 2003; Relas et al., 2001; Tammi et al., 2000).

The strategies used to express and report the results have widely varied, but in most studies, the plasma concentrations of fat-soluble vitamins and their precursors have been expressed after standardisation for either LDL-cholesterol or total cholesterol. Indeed, as discussed above, a decrease in plasma lipids may provide an explanation for the reduced levels of plasma fat-soluble antioxidants because plasma fat-soluble antioxidants are transported in part by lipoproteins (Mensink et al., 2002).

Most studies have reported results for carotenoids, and some have provided results for vitamins E and D (Clifton et al., 2004; Gylling et al., 2010; Gylling and Miettinen, 1999; Gylling et al., 1999; Kaffe et al., 2012; Korpela et al., 2006; Nguyen et al., 1999; Raeini-Sarjaz et al., 2002) and for vitamin A (Amundsen et al., 2004; Clifton et al., 2004; Gylling et al., 2010; Homma et

al., 2003; Judd et al., 2002; Korpela et al., 2006; Plat and Mensink, 2001; Raeini-Sarjaz et al., 2002) and vitamin K (Heggen et al., 2010; Korpela et al., 2006; Raeini-Sarjaz et al., 2002). Most commonly, standardised results have been reported for retinol, □-tocopherol, □-carotene, □-carotene, and □-cryptoxanthin; they have rarely been reported for vitamin K and other carotenoids and never for vitamin D and vitamin E.

Results

Among the 52 studies identified during the selection step (**Table 2**), we excluded the bioavailability studies (n=3), the studies in which food vectors were enriched in fat-soluble vitamins or their precursors (n=8), and the studies in which the diet was enriched with fruits and vegetables (n=3). The main results from the remaining 38 interventional studies are described below (see **Table 1** for details).

Phytosterol/stanol supplementation did not impact the plasma concentration of vitamins A, D and K, except in a few studies that reported a decrease by 16% (Plat and Mensink, 2001), 4% (Hendriks et al., 2003) and 14% (Hendriks et al., 2003), respectively (a significant reduction in vitamin K1 was also reported by Heggen et al. but the values were not provided (Heggen et al., 2010)). Phytosterol/stanol supplementation did not impact the plasma concentration of vitamin E. When considering the different forms of vitamin E separately, only tocopherols - and not tocotrienols - were studied. For □-tocopherols, 16 studies showed a median reduction of 10% (with a maximum of -16%) (**Table 2**). After standardisation, the changes in □-tocopherol were no longer significant. Similar results were shown for other tocopherols, with a reduction of 9%

(1 study), 12% (median of 2 studies), 10% (1 study) and 13% (NS) for □-, □-, □- and □+□-tocopherols, respectively, with no more significant changes after standardisation (**Table 2**).

In contrast, the results were much different for vitamin A precursors, namely □-, □- and □+□-carotenes and □-cryptoxanthin (**Table 2**). For □-carotene, the median change was -13% (9 studies, maximum -42%) without standardization and -18% (6 studies, maximum -33%) after standardisation. However, other studies did not report a significant effect (13 without standardization and 14 after standardisation, **Table 2**). The results for □-carotene were more marked compared with those for □-carotene: the median reduction was -24% (20 studies, maximum -74%) and -25% (8 studies, maximum -37%) after standardisation (**Table 2**). Fewer studies considered the sum of (□+□□□carotenes, but their results were consistent with those investigating □- and □-carotene separately. Finally, for □-cryptoxanthin, we observed a median reduction of -16% (5 studies, maximum -32%), and -9% after standardisation (2 studies, maximum -9%, **Table 2**). However, 9 studies also reported an absence of significant changes either before or after standardisation.

Standardisation therefore does not markedly change the median value of the reduction percentage for □-carotene, □-carotene and □+□-carotene, but it attenuates the reduction percentage for □-tocopherol (from -10% to +6%), □-tocopherol (from -12% to NS) and □-cryptoxanthin (from -16 to -9%, **Table 2**). The most striking result was the reduction in the median □-carotene level by -24% by phystoterols/stanols and by -25% after standardisation. Standardisation has a more marked effect on the reduction of maximal value changes.

Finally, it is likely that the effects of PAP on fat-soluble vitamin concentrations are dependent on several parameters, such as phytosterols *vs* phytostanols, vitamin- and carotenoid-enrichment of

food vectors, doses of PAP, daily consumption, the duration of follow-up and/or the nature of the accompanying diet and food vector. The potential influence of these factors is discussed below.

When considering phytosterols (n = 26 studies) and phytostanols (n = 14 studies) separately (results not shown), comparisons could only be conducted for □- and □-carotene due to the lack of studies investigating the other compounds. When the results were standardised, phytosterol supplementation led to a greater reduction in □- (median: -24% *vs* -15%, respectively) and □-carotene (median: -26% *vs* -21%, respectively) compared with phytostanol supplementation. Without standardisation, phytosterol supplementation also led to a greater □- (median: -36 *vs* -13%, respectively) and □-carotene (median: -39 *vs* -21%, respectively) reduction compared with phytostanol supplementation.

The studies involving test foods enriched with fat-soluble vitamins and carotenoids (Table 1) (Amundsen et al., 2004; Brufau et al., 2004; Granado-Lorencio et al., 2011; Hallikainen et al., 2000; Nestel et al., 2001; Quílez et al., 2003; Relas et al., 2001; Tammi et al., 2000) showed no change in plasma or serum levels and occasionally significant increases in □-tocopherols (Brufau et al., 2004; Granado-Lorencio et al., 2011; Nestel et al., 2001; Quílez et al., 2003) or retinol (Amundsen et al., 2004).

A study comparing the effects of 0.85, 1.62 or 3.26 g/day of phytosterols for 3.5 weeks reported no dose effect of the reduction in □+□-carotene (Hendriks et al., 1999 123286). Similarly, another study reported a decrease in α-tocopherol with 3 g/day of stanols but not with 2 g/d (Homma et al., 2003). In addition, Davidson et al. reported a dose-related effect on the decrease of □- and □-carotene with 9 g/day but not with 6 or 3 g/d. In contrast, two studies reported no difference with 1.5 and 3 g/day of phytosterols (Christiansen et al., 2001) and for 3, 6 or 9 g/day

of stanols (Mensink et al., 2010). Considering the scientific substantiation of a health claim related to 3 g/day plant sterols/stanols to lower blood LDL-cholesterol (EFSA Panel on Dietetic Products, 2012), based on the results shown in Table 1 (6 studies: range 2.5-3.9 g/day), the □-carotene level was reduced by ≈20% (median) for both standardised and non-standardised data.

To our knowledge, only one study has addressed the issue of the number of times PAP were consumed daily. Hydrocarbonated carotenoid reduction was more pronounced when the 2.5 g/day of stanols was administered as a single dose compared to when the dose was divided and taken 3 times in a day (Plat et al., 2000).

Regarding the duration of phytosterol use, Hendricks et al. observed an effect on the □-carotene/LDL-C and □-cryptoxanthin/LDL-C ratios over 52 weeks but not at 26 weeks, whereas this was not the case for the other parameters (Hendriks et al., 2003 123158). However, most studies were shorter (median = 8 weeks). Among the studies that were conducted over 3 months or more, two studies reported a decrease in □-carotene following the consumption of phytosterols (Gylling et al., 1999; Hernández-Mijares et al., 2010) and two did not report any significant effect (Bañuls et al., 2010; Christiansen et al., 2001). Therefore, it is not possible to draw any conclusions from those data.

Another important parameter to consider is the diet consumed by the subjects during the study. According to the study reports, subjects were instructed to maintain their usual diet or to follow a "healthy" diet or a diet enriched in fruits and vegetables, although the diets followed were often poorly characterised. One study reported a decrease in β-carotene following the consumption of phytosterols when subjects consumed their usual diet but not when subjects in a parallel group were instructed to consume a "healthy" diet (Hernández-Mijares et al., 2010). This result was

confirmed by a report that additional intake of fruits and vegetables alleviates the reduction in plasma concentrations of β-carotene (Noakes et al., 2002) or results in an increase in plasma β-carotene (Amundsen et al., 2002; Clifton et al., 2004). However, in another study, no protective effect of a fruit and vegetable-rich diet was observed; however, the study used a very high level of plant sterols (6.6 g/d) and the authors hypothesised that the subjects failed to increase their intake of fruits and vegetables up to the recommendations of the study (Clifton et al., 2004).

Because margarine is the predominant food vector, it is not possible to infer any potential effect of the food vector used to deliver phytosterols/stanols.

Standardisation of the results

In the majority of studies reported in the literature, the results obtained for plasma or serum concentrations of fat-soluble vitamins and their precursors were expressed after standardisation or adjustment for LDL cholesterol, total cholesterol or triglycerides (Heggen et al., 2010) (**Table 1**). Indeed, according to Mensink *et al*., "Because plasma lipid-soluble antioxidants are transported by lipoproteins, a decrease in plasma lipids may simply be the cause of the decreased plasma lipid-soluble antioxidant concentrations. Therefore, concentrations are generally standardised for a plasma lipid fraction, but no uniformity exists" (page 212) in the way the results are expressed (Mensink et al., 2002).

There may be another reason for this standardisation, which, to our knowledge, has never been mentioned in the literature: to limit the inter-individual variability due to significant differences in cholesterol concentrations among individuals within the same study.

Otherwise, the standardisation may also result in an overcorrection because fat-soluble vitamins are not equally transported by all lipoproteins and the relationship may not be linear. Following standardisation, the decrease in the concentration of fat-soluble vitamins was often no longer significant. Thus, it could be argued that standardisation tended to mask the effect of PAP on decreased fat-soluble vitamin concentrations. However, even if the effect size estimate tended to be reduced after standardisation, decreases in carotenoids concentration remained significant in most studies.

Bioavailability studies

Interestingly, bioavailability studies have confirmed the results obtained in clinical trials, particularly for α-tocopherol and □-carotene, which suggests that standardisation of result may indeed lower the effect size of PAP on some fat-soluble vitamins and carotenoids.

Only three studies have evaluated the effect of PAP on the bioavailability of fat-soluble vitamins in humans. The first study was conducted in children and parents with familial hypercholesterolaemia who consumed a test meal that was rich in retinol, α-tocopherol, and fat supplemented with 1 g ester stanols (Amundsen et al., 2004). The results showed that ester stanols did not change the postprandial 24-h area under curve for vitamins A and E or for □-carotene. The second study was conducted in normocholesterolemic subjects who received 2.2 g/d phytosterols (added to skim milk) for one week (Richelle et al., 2004). Free and ester sterols reduced the bioavailability of β-carotene by ~50% and of □-tocopherol by ~20%, but the reduction in □-carotene bioavailability was significantly less with plant-free sterols than with

plant sterol esters. In the third study, 36 volunteers consumed a fruit milk drink enriched with β-cryptoxanthin with or without phytosterols (2 g/day) for 4 weeks (Granado-Lorencio et al., 2011). In this adequately powered study, the addition of phytosterols had no effect on the *in vivo* bioavailability of β-cryptoxanthin and on the *in vitro* bioaccessibility, as measured with a digester that modelled gastrointestinal digestion.

Conclusions from interventional studies

Many experimental parameters can influence the effect of PAP on concentrations of fat-soluble vitamins. The majority of studies reported a median decrease in the concentration of □-carotene by approximately 24% (-39% for phytosterols and -21% for phytostanols). The explanation for the 2-fold difference between phytosterols and phytostanols remains unclear but might be attributed to a lower interaction of phytostanols with micellar absorption. These results are supported by the only study of bioavailability of □-carotene that used radiolabelled tracers and showed a decline in absorption due to the daily intake of phytosterols. However, due to the important number of studies that showed no effect on □-carotene levels (n = 13 when results are not standardised and n = 15 when results are standardised; **Table 2**), additional studies are needed to estimate more precisely the average impact of plant sterols on plasma □-carotene and to infer effects on other carotenoids.

Based on the current literature, PAP do not appear to have a large impact, if any, on other fat-soluble vitamins. This observation could be easily explained by differences in the mechanisms of absorption and transport of the different fat-soluble vitamins and carotenoids (Borel et al., 2009

18244). This updated analysis is therefore in line with the meta-analysis by Katan et al. reported in 2003 with namely no significant change in retinol and vitamin D, -20% change in β-carotene and -9% change in α-carotene (Katan et al., 2003).

Thus, to definitively answer the question of whether consumption of products enriched with PAP actually decreases the bioavailability of fat-soluble vitamins, much more dedicated and specific bioavailability studies are required. Accordingly, it should be recognised that the results reported in virtually all of the reported studies provide only a rough, inaccurate assessment of the impact of PAP on fat-soluble vitamin status. However, considering the total evidence, plant sterols result in a decrease in the status of carotenoids, as evidenced by the decrease in plasma concentrations irrespective of the way to report the results (Table 2).

PLASMA CAROTENOID REDUCTION AND CARDIOVASCULAR RISK

As the main and most obvious conclusion is that consumption of PAP lowers plasma carotenoid levels, we elected to evaluate the possible health impact of such an adverse effect for □-carotene alone. Because PAP are consumed with the objective to lower cardiovascular risk, the relation between plasma carotenoid levels and cardiovascular risk warrant specific mention. Although plasma carotenoids level is not established as a risk factor for cardiovascular diseases, plasma levels of carotenoids have been shown to be inversely associated with cardiovascular risk (Kohlmeier and Hastings, 1995; Kritchevsky, 1999; Palace et al., 1999; Voutilainen et al., 2006).

Similarly, the consumption of carotenoid-rich fruits and vegetables has been associated with a reduced risk of cardiovascular disease (Gaziano et al., 1995).

The LRC-CPPT Study showed that the risk of coronary heart disease was 38% lower (and 72% lower when considering never-smokers) in the highest quartile of plasma carotenoid concentration compared to the lowest quartile (Morris et al., 1994). Kritchevsky *et al*. showed in 12,773 participants of the Atherosclerosis Risk in Communities Study that those in the highest quintiles of consumption of carotenoids had a lower prevalence of atherosclerotic plaques (-25% in women and -36% in men) (Kritchevsky et al., 1998). The Women's Health Study showed a 33% reduction in the risk of cardiovascular disease in the highest quartile of plasma carotenoids compared to those in the lowest quartile (Sesso et al., 2004). The SENECA study showed that plasma □-carotene concentrations were associated with a lower mortality risk (adjusted RR for an increment of 0.39 □mol/L: 0.79; 95% CI: 0.70, 0.89) (Buijsse et al., 2005). In Japanese men and women, high serum levels of carotenoids and provitamin A activity were associated with a reduction in the risk of mortality due to cardiovascular diseases (Ito et al., 2006a; Ito et al., 2006b). In addition, significantly lower plasma concentrations of β-cryptoxanthin were observed in patients with coronary artery disease compared with controls (Lidebjer et al., 2007). More recently, the CARDIAC study in 300 Scottish men and women at high risk of coronary heart disease reported that the levels of blood total carotenoids were very low (mean values = 0.18 ± 0.13 □g/mL) and significantly associated with coronary risk (Miyashita et al., 2011).

Therefore, although plasma β-carotene is not considered a risk factor for cardiovascular diseases and could, as a marker of a healthy diet, be only non-causally associated with CVD risk, the size

of the decrease in plasma carotenoids (~24%) following the consumption of PAP is in the same range as that associated with modification of cardiovascular risk in the congruent literature.

Finally, it is worth mentioning that this size of plasma □-carotene reduction (~24%) may be also compatible with potential increased risk of cancers as exemplified by some results from other observational studies. For example, in 27,084 male smokers aged 50-69 years, lower risks of lung cancer were observed for the highest versus the lowest quintiles of serum □-carotene (RR = 0.81 ; IC = 0.69, 0.95 for quintile Q5; P_{trend} = 0.02) (Holick et al., 2002). In 2006, in 3254 Japanese subjects aged 39-85 (men and women), high serum values of carotenoids (including xanthophylls) were reported to be apparently associated with low hazard ratios for mortality rates of cancer of all sites (HR = 0.76 (0.60-0.96) ; p = 0.023), especially liver (HR = 0.38 (0.17-0.88) ; p = 0.023) and colorectal (HR = 0.36 (0.18-0.73) ; p = 0.005) cancers (Ito et al., 2006). And more recently, in a pooled analysis of eight prospective studies including 3055 case subjects and 3956 matched control subjects, statistically significant inverse associations with breast cancer were observed for □-carotene (RR 0.83, 95% CI 0.70 to 0.98, P_{trend} = 0.02), suggesting that women with higher circulating levels of □-carotene may be at reduced risk of breast cancer (Eliassen et al., 2012).

GENERAL CONCLUSIONS

There is good scientific evidence that regular consumption, especially over the long-term, of foods fortified with PAP (as recommended in the labelling), especially with phytosterols, reduce

plasma β-carotene. This decline can be mitigated or offset by an increase in the consumption of fruits and vegetables. Indeed, at the recommended dose of 3 g/day, the observed reduction was ~20%. Furthermore, an increased cardiovascular risk was observed for differences in plasma □-carotene concentrations of the same magnitude as the estimated average decrease in response to PAP consumption. However, data are still lacking regarding the impact of PAP on other fat-soluble vitamins and, more precisely, concerning the extent of the reduction of plasma carotenoids, warranting dedicated bioavailability studies.

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ABBREVIATIONS

PAP = Phytosterols and phytostanols

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Table 1. **Human interventional studies for the effects of phytosterols/stanols-enriched foods on serum or plasma carotenoid and fat-soluble vitamin concentrations.**

Reference	Subject status	Age range (mean values) (yrs)	Number of subjects	Study design	Duration of the study	Food vector (daily dose)	Diet	Standardized results	Not standardized results
Weststrate *et al*. 1998 (Weststrate et al., 1998)	Normochol./mildly hypercholl.	45 ±12.8	95	Randomized double-blind placebo-controlled balanced incomplete Latin square, parallel	3.5 wks	Margarines (Benecol-sterols, soybean oil, rice bran oil or sheanut oil, 1.5-3.3 g)	Regular	↘ □+β-carotene (19, 19, 9 and 43%, respectively)	↘ □+β-carotene (22, 23, 8 and 43%, respectively)
Gylling *et al*.1999 (Gylling et al., 1999)	Moderately hypercholl.	25-64 (50 ±1 for control, 51 ±1 for test)	102 (cases) + 49 (controls)	Randomized double-blind, parallel	1 yr + 2 months	Margarine (3 g sistostanol)	Regular	No change for α-tocopherol and α-carotene ↘ β-carotene (25 %)	No change for retinol ↘ α-tocopherol (10%) ↘ α-carotene ↘ □-carotene ↗ 25-hydroxyvitamin D

									(14%) NB: changes in retinol, □-tochopherol and □-carotene concentrations are correlated with cholesterol concentration changes; when stopping phytostanols supplementation, □- and β-carotene concentrations increase
Hallikainen and Uusitupa 1999 (Hallikainen	Hypercholesterolemic	20-60 (43.2 ±8.2/wood, 40.8 ±9.3/vegetable oil, 46.0 ±8.2/con	18 (wood esterified stanols) + 20	Randomized double-blind, parallel	4 wks run-in (high-fat) + 8 wks	Light margarine (2.34 g wood stanols or	Low-fat/cholesterol	No change for □-carotene ↗ α-tocopherol (8	No change for retinol ↘ □-carotene (27 and

and Uusitupa, 1999)		trol)	(vegetable oil esterified stanols) + 17 (controls)			2.20 g vegetable oil stanols)		and9%, respectively)	27%, respectively) ↘ α-tocopherol (12 and 8%, respectively)
Hallikainen *et al*. 1999 (Hallikainen et al., 1999)	Hypercholesterolemic	43.2 ±8.2 (wood), 40.8 ±9.3 (vegetable oil) and 46.0 ±8.2 (control)	18 (wood esterified stanols) + 20 (vegetable oil esterified stanols) + 17 controls	Randomized double-blind, parallel, placebo-controlled	4 wks run-in (high-fat) + 8 wks	Light margarine (2.34 g wood stanols or 2.20 g vegetable oil stanols)	Low-fat/cholesterol	No change for α- and β-carotenes	No change for α-carotene ↘ β-carotene (27 and 74%, respectively)
Hendriks *et al*. 1999 (Hendriks et al., 1999)	Normocholesterolemic ou hypercholesterolemic. Moderately	19-58 (37 ±10)	100	Double-blind, placebo-controlled, balanced incomplete Latin square	3.5 wks	Margarines (0.85, 1.62 or 3.26 g phytosterols)	Apparently regular	No change for □+□-carotene (for 1.62 g) and □-tocopherol ↘ □+β-carotene (10 and 30% à	No change for □-tocopherol (for 0.85 g), vitamin K1 and 25-OH-vitamine D ↘ □+β-carotene (12, 9 and

								0.85 and 3.26 g, respectively)	30%, respectively) ↘ □-tocopherol (8 and 9% à 1.62 and 3.26 g, respectively)
Gylling *et al*. 1999 (Gylling and Miettinen, 1999)	Healthy post-menopausal women	50-55 (52.7 ±1.2)	23	Randomized double-blind, cross-over	6 wks	Rapeseed oil margarine and butter (3.18/3.16 g stanol/25 g margarine, and 2.43 g stanol/25 g butter)	Regular	No change □-tocopherol ↘ □-carotene (14% for sitostanol ester-rich margarine, 29% for campestanol ester-rich margarine and 29% for sitostanol ester butter), and	No change for 25-hydroxyvitamin D (margarine) and retinol ↘ □-tocopherol (9% for sitostanol ester-rich margarine, 8% for campestanol ester-rich margarine and 5% for sitostanol ester butter), □-

								β-carotene (30% for sitostanol and campestanol ester-rich margarine, and 22% for sitostanol ester butter)	carotene (30% for sitostanol ester-rich margarine, 33% for campestanol ester-rich margarine and 30% for sitostanol ester butter), and β-carotene (33% for sitostanol and campestanol ester-rich margarine, and 27% for sitostanol ester butter) ↗ Vitamine D (24%/butter)

Nguyen *et al*. 1999 (Nguyen et al., 1999)	Healthy	≥ 20 (53 ±11)	318	Randomized, double-blind, parallel	8 wks	European or US margarine-like spreads containing stanol ester (1 or 0.67 g/8 g spread x 3)	Regular	↘ □-carotene (25% for European margarine at 1 g, 27% for US margarine at 1 g and 20% for US margarine at 0.67 g)	No change for 25-hydroxyvitarnin D and vitamin A
Andersson *et al*. 1999 (Andersson et al., 1999)	Moderately hypercholesterolaemic	30-65	28 (men) + 33 (women)	Randomized, double-blind, parallel	8 wks	Low-fat stanol ester-containing margarine	Strictly controlled lipid-lowering diet	-	No change for vitamins A and D, □- and □-tocopherol, and α- and □-carotene
Hallikainen *et al*. 2000 (Hallikainen et al., 2000)	Moderately hypercholl.	30-65	20 (men) + 22 (women)	Randomized double-blind repeated measures design with three test spreads	4 wks	2 vitamin A/D-enriched margarines (wood	Low-fat	No change for □-carotene, □-carotene, and □- and □-tocoph	No change for retinol, □-carotene, □-tocopherol and 25-OH-

						sterols - STANOL: 2.02 g, and plant oil - STEEST: 2.02 g)		erols	vitamin D ↘ β-carotene (12 and 17%, respectively) ↘ α-tocopherol (8 and 7%, respectively)
Plat and Mensink 2000 (Plat and Mensink, 2000)	Healthy	18-65 (33 ±15)	41 (men) + 71 (women)	Randomized double-blind, parallel	8 wks	Margarine (3.8 g phytostanols from plant oil or pine wood)	Regular	-	No change for factor VII-depending vitamin K activity
Plat *et al*, 2000 (Plat et al., 2000)	Normochol. or moderately hypercholl.	18-65 (31 ±14)	39	Randomized double-blind, placebo-controlled, cross-over	4 x 2 wks	Margarine or shortenings (2.5 g stanols one or three times)	Regular	No change for α-, □- and □+□-tocopherols, β-cryptoxanthin and α- and β-carotenes	Once a wk: No change for retinol, □- and □+□-tocopherols, β-cryptoxanthin and α-carotene

								Total hydrocarbonated carotenoids: NS for 3 times/wk and ↘ for 1 time/wk	↘ α-tocopherol (4%) ↘ β-carotene (19%) Three times/wk: No change for retinol, □-tocopherols and α-carotene ↘ α-tocopherol (7%) and □+□-tocopherol (13%) ↘ β-carotene (22%)
Tammi *et al*. 2000 (Tammi et al., 2000)	Moderately hypercholl.	6	66	Randomized double-blind, placebo-controlled, cross-over	2 x 3 months	Vitamin A/D-enriched margarine (1.6 g phytostanols)	Apparently regular	No change for α-tocopherol ↘ β-carotene (13%)	No change for vitamins A and 25-OH-vitamin D ↘ α-tocopherol

									(6%) ↘ β-carotene (27%)
Volpe *et al.* 2001 (Volpe et al., 2001)	Moderately hypercholl.	33-69	30	Randomized single-blind, cross-over	8 wks	Yogurt-based beverage (2 g sterols)	Low-fat (≤ 30% energy)	-	No change for vitamins A and E ↗ vitamine D (18%)
Nestel *et al.* 2001 (Nestel et al., 2001)	Hypercholl.	34-70 (60 ±9)	22	Randomised, controlled, single-blind, cross-over	4 wks	Tocopherol-enriched margarine, breakfast cereals and bread (2.4 g)	Apparently regular	-	No change for α- and β-carotenes and β-cryptoxanthin ↗ □-(14%) and □-(59%) tocopherols
Christiansen *et al.* 2001 (Christiansen et al., 2001)	Hypercholl.	25-64 (50.7)	55 (men) + 100 (women)	Randomized double-blind, placebo-controlled, parallel	6 months	Margarine (1.5 or 3.0 g phytosterols)	Regular	-	No change for retinol, □-tocopherol, □- and □-carotene
Maki *et al.* 2001 (Maki	Moderately hypercholl.	21-75	119	Randomized, double-blind, 3-	5 wks	Margarine (1.1 or 2.2	NCEP-I (« prudent » diet,	No change for □-carote	No change for retinol,

et al., 2001)				group parallel, controlled		g esterified phytosterols)	from National Cholesterol Educational Program)	ne and cryptoxanthin ↘ trans-β-carotene (17 and 24%, respectively)	□- and □-tocopherol, 25-OH-vitamine D, phylloquinone (vitamine K1), □-carotene at 1.1 g) and cryptoxanthin ↘ trans-β-carotene (22 and 26%, respectively) ↘ □-carotene (22% at 2.2 g)
Davidson *et al*. 2001 (Davidson et al., 2001)	Healthy	18-65	84	Randomized, double-blind, controlled, parallel	8 wks	Margarine and light salad seasonings (3, 6 or 9 g phytosterols)	Regular	No change for □- and □-tocopherols, trans-□-carotene (for 3 and 6 g), □-carote	No change for retinol, 25-OH-vitamine D, □- and □-tocopherols, vitamine K1 (phylloquinone

								ne and crypto xanthin ↘ trans- □- carote ne (17% for 9 g)	), trans- □- caroten e (for 3 and 6 g), □- caroten e (for 3 and 6 g) and cryptox anthin ↘ trans- □- caroten e (26% for 9 g) ↘ □- caroten e (25% for 9 g)
Plat and Mensi nk 2001 (Plat and Mensi nk, 2001)	Normoch ol.	33 ±16	34 (woo d) + 36 (plan t oil) + 42 (cont rols)	Randomi zed, double- blind, controlled , parallel	8 wks	Marg arine (woo d esteri fied stanol s, 4 g, or plant oil stanol s, 3.8 g)	Regular	No change for total and hydroc arbona ted carote noides, total tocoph erols, α- and β- carote ne, β- crypto xanthi n and α-, □- and	No change for retinol, β- caroten e (wood stanols only), α- caroten e, β- cryptox anthin, □+□- tocophe rol an □- tocophe rol (wood

								□+□-tocopherols ↘ retinol (16 and 12%, respectively)	stanols only) ↘ α-tocopherol (approximately 8 and 6%, respectively), β-carotene (approximately 25%, plant oil stanols only) and □-tocopherol (approximately 10%, plant oil stanols only) NB: changes for hydrocarbonated carotenoids concentrations are significantly

									correlated with cholesterol absorption reduction; changes for single carotenoides and tocopherols are not
Relas *et al*. 2001 (Relas et al., 2001)	Healthy	52 ±5	10	Post-prandial	24 hrs	Test fat-rich meal ±1 g esterified phytostanols and supplemented with retinol (0.9-3.7 mg), α-tocopherol (70-581 mg) and β-carote	Fat tolerance test (*i .e.*, provoked hyperlipidemia)	-	Area under the curve percent changes for β-carotene, retinyl palmitate and □-tocopherol are not significant

						ne (25-150 mg)			
Amundsen *et al.* 2002 (Amundsen et al., 2002)	Familial history of hypercholestérolemia	7-12 (10.5 ±1.7)	38	Randomized, double-blind, crossover	8 wks	Margarine (1.6 g phytosterols)	Cholesterol and saturated fat reduction; unsaturated fat, fruit and vegetable increase	No change for □- and □-carotene ↗ retinol (10%) ↗ □-tocopherol (3%)	No change for □-tocopherol and □-carotene ↗ retinol (9%) ↘ □-carotene (19%)
Raeini-Sarjaz *et al.* 2002 (Raeini-Sarjaz et al., 2002)	Hypercholesterolemia	37-61	15 (men)	Randomized, double-blind, placebo-controlled cross-over	3 wks	Margarine (1.92 g esterified phytosterols or 1.76 g esterified phytostanols)	Balanced	No change for □- and □-tocopherols, □- and β-carotenes	No change for retinol, □- and □-tocopherols, vitamins D and K, □- and □-cryptoxanthin, and □- and β-carotene NB: short study duration; the balanced diet has led

									to carotenoids increase over 21 days in each group
Judd *et al*. 2002 (Judd et al., 2002)	Moderately hypercholl.	25-65	26 (men) + 27 (women)	Randomized, double-blind, cross-over	3 wks	Salad seasoning (3.6 g sterols)	Isocaloric balanced diet (32% energy from lipids)	-	No change for retinol, □- and □-tocopherols, □-cryptoxanthin and β-cryptoxanthin in men ↘ total carotenoids (10 %) ↘ α- (13 %) and β-carotene (13 %) ↘ β-cryptoxanthin (14 %) in women only NB: significant β-caroten

									e, α-carotene and β-cryptoxanthin reductions (only women) are not associated with plasma lipid changes
Mensink *et al*. 2002 (Mensink et al., 2002)	Normochol.	18-65 (36 ±14)	60	Randomized, double blind, placebo-controlled, parallel	4 wks	Low-fat yogurt (3 g stanols)	29% lipids	LDL-Cholesterol standardization : No change for β-cryptoxanthin, □-carotene and hydrocarbonated carotenoids ↗ total (9%), α- (8%), □- (25%) and □+□-	No change for retinol, and α-, □- and □+□-tocopherols ↘ β-cryptoxanthin and □- and β-carotenes NB: β-carotene reduction is not limited to LDL fraction ; β-

								(25%) tocopherols ↘ β-carotene (14%) Total cholesterol standardization ±triglycerides : same results than with LDL-Cholesterol standardization except: ↘ β-cryptoxanthin, □-carotene and hydrocarbonated carotenoids	carotene has been measured in all lipoproteins and has been decreased in each one
Noakes *et al*. 2002 (Noakes et al., 2002)	Hypercholesterolemic	20-75 (58 ±8)	46	3-way, double-blind, randomized cross-over	3 wks	Margarine (2.3 g phytosterols or	Carotenoid-rich (≥ 5 fruits and vegetabl	No change for retinol, α-tocoph	-

						2.5 g phytostanols)	e servings)	erol and □- and □-carotenes	
Ntanios *et al*. 2002 (Ntanios et al., 2002)	Healthy	24-67 (45)	53	Double-blind, cross-over	3 wks	Margarine (1.8 g phytostanols)	Regular	↘ □-carotene	No change for vitamins A and E ↘ □-carotene (21%)
Quilez *et al*. 2003 (Quílez et al., 2003)	Normochol.	Data not shown	57 (29/control+ 28/test)	Randomized, double-blind, placebo-controlled, repeated-measures	8 wks	Croissants and □-tocopherol/□-carotene-enriched Spanish muffins (*magdalenas*) (3.3 g)	Regular	No change for □-tocopherols, □- and β-carotenes ↗ □-tocopherol (9%)	No change for □-tocopherols, □- and β-carotenes ↗ □-tocopherol (8%)
Homma *et al*. 2003 (Homma et al., 2003)	Healthy	≥ 20	105	Randomized, parallel, placebo-controlled	4 wks	Margarine (2 or 3 g phytostanols)	Low-lipid and cholesterol	-	No change for retinol and □-carotene ↘ α-tocopherol (6%

									with 3 g)
Hendriks *et al*. 2003 (Hendriks et al., 2003)	Healthy	35-64 (48 ±7-8)	185	Randomized, double-blind placebo-controlled, parallel	1 yr	Margarine (1.6 g phytosterols)	Regular	At 26 wks, compared to control, no change for □-carotene, □-cryptoxanthin and □-tocopherol At 52 wks, compared to control, no change for □-tocopherol ↘ □-carotene (14% at 26 and 24% at 52 wks) ↘ □-carotene (15% at 52 wks) ↘ □-	At 26 and 52 wks, compared to control, no change for retinol ↘ □-carotene (22% at 26 and 24% at 52 wks) ↘ □-carotene (11% at 26 and 14% at 52 wks) ↘ □-cryptoxanthin (3% at 26 and 10% at 52 wks) ↘ □-tocopherol (3% at 26 and 2% at 52 wks) ↘ 25-OH-vitamin

								cryptoxanthin (9% at 52 wks)	e D (17% at 26 and 4% at 52 wks) ↘ vitamine K1 (14% at 26 wks)
Colgan *et al*. 2004 (Colgan et al., 2004)	Hypercholesterolemic	44.1 ±7.6 (men) 48.5 ±9.8 (women)	48	Randomised, placebo-controlled, cross-over double-blind	3 wks	Light margarine (1.6 g phytosterols)	NCEP-I (« prudent » diet, from the National Cholesterol Educational Program)	No change for □-carotene	No change for retinol, α- and □-tocopherol, □-carotenes and □-cryptoxanthin ↘ □-carotene (13%)
Thomsen *et al*. 2004 (Thomsen et al., 2004)	Hypercholesterolemic	45-65	71	Double-blind, randomized, placebo-controlled three-arm cross-over	4 wks	Milks (1.2 or 1.6 g non esterified and hydrogenated sterols)	Regular	No change for α-tocopherol, □- and □-carotenes and □-cryptoxanthin	No change for □-cryptoxanthin ↘ α-tocopherol (5 and 7%, respectively) ↘ □-carotene (21% for 1.6 g) ↘ □-

									carotene (13 and 13%, respectively) ↗ □-carotene (17% for 1.2 g)
Brufau *et al*. 2004 (Brufau et al., 2004)	Normocholest.	Data not shown	57 (29/control + 28/test)	Double-blind	8 wks	Croissants and □-tocopherol/□-carotene-enriched Spanish muffins (*magdalenas*) (3.3 g)	Regular	-	No change for □-carotene ↗ □-tocopherol (8%)
Richelle *et al*. 2004 (Richelle et al., 2004)	Normocholest.	29 ±1	26 (men)	Randomized, double-blind, cross-over (+ experimental absorption study)	1 wk	Skimmed milk (2.2 g esterified or free phytosterols)	Standard	-	↘ β-carotene bioavailability (48% with free sterols and 57% with esterifie

									d sterols) ↘ α-tocopherol bioavailability (no reduction with free sterols and 27% with esterified sterols) ↘ retinyl palmitate bioavailability (32% with free sterols and 48% with esterified sterols) NB: reduction with esterified sterols is higher than with

									free sterols; β-carotene bioavailability reduction is significantly less with free sterols than with esterified sterols
Clifton *et al*. 2004 (Clifton et al., 2004)	Moderately hypercholl.	20-75 (55.3)	23 (women) + 12 (men)	Nonrandomized, single blind, parallel	6 wks with phytosterols + 6 wks with phytosterols, fruits and vegetables	Bread, breakfast cereals or margarine (6.6 g phytosterols)	Regular ± fruits and vegetables	No change for retinol (at 6 wks), α-carotenes (at 12 wks) and β-carotene (at 12 wks) ↘ retinol (6% at 12 wks) ↘ α-carotene	No change for retinol, □-tocopherol (at 6 wks), 25-hydroxyvitamin D, □-carotene and □-carotenes (at 12 wks) ↘ α-tocopherol (14% at 12 wks) ↘ □-

								(20% at 6 wks) ↘ β-carotene (26 at 6 wks)	carotenes (29% at 6 wks)
Amundsen *et al*. 2004 (Amundsen et al., 2004)	Hypercholesterol. (parents take statins)	7-13 (9.6 ±1.5) (children) 32-51 (42.9 ±5.2) (parents)	37 20	Open-label follow-up, controlled cross-over	26 wks	Tocopherol and retinol-enriched margarine (1.76 g phytosterols)	Regular	No change for □-tocopherol (children), □-carotene and □-carotenes (children and parents) ↗ retinol (12% for children and 13% for parents) ↗ α-tocopherol (8% for parents)	No change for retinol, □-tocopherol, □-carotene and □-carotene (children and parents)
Polagruto *et*	Hypercholesterol.	49 ±2 (control)	22 (men	Randomized,	6 wks	Flavanol-	Regular	No change	No change

al. 2006 (Polagruto et al., 2006)		56 ±2 (test)	) + 48 (women)	double-blind, placebo-controlled, parallel-arm		enriched chocolate bar (1.5 g phytosterols)		for retinol, □-tocopherol, □-carotene and □-cryptoxanthin ↘ β-carotene (16%)	for retinol, □-tocopherol, □-carotene and □-cryptoxanthin ↘ β-carotene (16%)
Devaraj *et al*. 2006 (Devaraj et al., 2006)	Healthy	19-74 (44 ±14/test and 48 ±15/control)	72	Randomized,placebo-controlled, double-blind, parallel	8 wks	Low-calorie orange juice (2 g phytosterols)	Regular	-	No change for □-tocopherol, □-carotene and □-carotene
Korpela *et al*. 2006 (Korpela et al., 2006)	Mildly hypercholl.	57 ±9 (57.6 ±9.1/test and 57.0 ±8.4/control)	164 (82/control + 82/test)	Randomized,double-blind, parallel	6 wks	Yogurt or low-fat cheese (2 g phytosterols)	Without restriction	No change for β-carotene, α- and □-tocopherols	No change for □-tocopherols, retinol, vitamin K1 and 25-OH-vitamin D ↘ α-tocopherol (8%, significant/control)

									↘ β-carotene (3%, significant/control)
Hansel *et al*. 2007 (Hansel et al., 2007)	Hypercholesterol. (± statins)	18-75 (49.5 ±13)	191	Randomized, double-blind, multicenter (5 centers), parallel	6 wks	Skimmed and fermented milk (1.6 g phytosterols)	General nutritional recommendations for moderate hypercholesterolemia	No change for □-carotene	↘ β-carotene (9%)
De Jong *et al*. 2008 (De Jong et al., 2008)	Hypercholesterol. (with statins)	18-65 (57.8 ±5.8/control, 58.4 ±9.9/sterols and 58.7 ±7.8/stanols)	45	Randomized, double-blind, placebo-controlled, parallel	16 wks	Margarine (2.5 g phytosterols or phytostanols)	35-36 % energy from fat	No change for hydrocarbonated carotenoids, total tocopherols, cryptoxanthin, and □- and β-carotenes	No change for antioxidant status and oxidative stress markers
Sanchez-Muniz *et al*. 2009 (Sanchez-Muniz et al.,	Hypercholesterol. with different ApoE genotypes	21-75 (57.5 ±10.2/□2/□3ApoE isoform, 59.0 ±10.4/□	207	Randomized, double-blind, parallel	5 wks	Margarine (1.1 g phytosterols or 2.2 g phytostanol	NCEP-I (« prudent », from National Cholesterol Educational	-	No change for vitamin K, tocopherols and cholecalciferol

2009)		□3/□3A poE isoform and 57.4 ±11.5/ □3/□4 plus □4/□4 ApoE isoform)				s)	Program)		(25-hydroxyvitamin D) ↘ □-caroten e (13%/c ontrol) ↘ cryptox anthin (32%/c ontrol) ↘ trans-□-caroten e (16%/c ontrol)
Tuomil ehto *et al*. 2009 (Tuom ilehto et al., 2009)	Moderat ely hypercho l.	27-74	52 (wo men) + 19 (men)	Randomi zed, double-blind, placebo-controlled , parallel	15 wks (3 x 5 wks)	Wood phyto sterol s-enric hed foods (1.25 g/5 wks, 2.5 g/ 5 wks, 5 g/5 wks)	Regular	No change for α-tocoph erol	No change for retinol and □-caroten e ↘ α-tocophe rol (10%)
Chen *et al*. 2009 (Chen et al., 2009)	Moderat ely hypercho l.	51.7 ±2.4	13 (men) + 9 (wo men)	Randomi zed, double-blind, cross-over	4 wks	Marg arine (3.3 g sterol s)	Typical America n	No change for □-, □- and □-tocoph erols ↗	No change for retinol and □-tocophe rol ↘ □-

								retinol (7%) ↘ □-(6%) and □-(8%) cryptoxanthins ↘ □-(14%) and □-(21%) carotenes	(8%) and α-(12%) tocopherols ↘ □-(16%) and □-(16%) cryptoxanthins ↘ □-(20%) and □-(28%) carotenes
Bañuls *et al*. 2010 (Bañuls et al., 2010)	Moderately hypercholl.	24-69 (50.0 ±10.2)	40	Randomized parallel	3 months	Skimmed milk (« low-fat ») (2 g phytosterols)	Standard « healthy »	-	No change for □-carotene ↘ cryptoxanthin (29%)
Mensink *et al*. 2010 (Mensink et al., 2010)	Moderately hypercholl.	18-70 (56 ±10)	49 (men) + 44 (women)	Randomized, double-blind, parallel	4 wks	Margarines and soya yogurt (3, 6 or 9 g phytostanols)	Regular	No change for α-tocopherol and □-carotene	↘ α-tocopherol (5.5, 6.9 and 3.8%, respectively) ↘ β-carotene (13.3, 4.0 and 7.3%, respectively)
Gylling *et al*.	Moderately	18-75 (61	49	Randomized,	10 wks	Margarine	Regular	No change	No change

2010 (Gylling et al., 2010)	hypercholl.	±1.5)		double-blind, placebo-controlled, parallel		and oat-based beverage (8.8 g phytostanols)		for α- and □-tocopherol ↘ α-carotene (33%) ↘ β-carotene (37%)	for vitamin A, □-tocopherol and 25-OH-vitamin D ↘ α-carotene (42%) ↘ β-carotene (47%) ↘ α-tocopherol (16%)
Hernández-Mijares *et al*. 2010(Hernández-Mijares et al., 2010)	Moderately hypercholl.	35-71 (50)	25 (men) + 59 (women)	Randomised, parallel trial with a three-arm design	3 months	Low-fat milk (2 g phytosterols)	« Healthy » or « free »	↘ β-carotene (« free » diet) ↗ β-carotene (« healthy » diet)	No change for cryptoxanthin (« free » and « healthy » diets) and □-carotene (« healthy » diets) ↘ β-carotene (21%, « free » diet)
Heggen *et al*. 2010 (Hegge	Hypercholl.	25-75 (52 ±12)	44 (men) + 15	Randomized, double-blinded,	4 wks	Margarine (2 g phyto	Regular	No change for □-, □- and	No change for □-tocophe

n et al., 2010)			(women)	cross-over		sterols from rapeseed or rosin oils)		□-tocopherols and vitamin K1 (phylloquinone) ↘ α-(15%) and □-(11%) carotenes ↘ α-tocopherol (4%)	rol ↘ α-(17%) and □-(18%) carotenes ↘ □-(11%), □-(9%) and □-(12%) tocopherols ↘ vitamin K1 (phylloquinone)
Granado-Lorencio *et al*. 2011 (Granado-Lorencio et al., 2011)	Apparently healthy	55	36	Randomized, cross-over for each subject	4 wks	□-cryptoxanthin-enriched milk-based fruit beverage	Fruits, vegetables, juices and beverages that are rich in measured compounds have been avoided	-	No change for □-cryptoxanthin
Sôderholm *et al*. 2012 (Soderholm et al., 2012)	Normocholest.	34.6 ±11.7 (test) 37.1 ±12.4 (control)	63	Double-blind, parallel	4 wks	Rye bread (2 g then 4 g phytosterols)	Regular	No change for □- and □-tocopherols, and □- and □-carote	No change for □- and □-tocopherols, and □- and □-caroten

								nes	es
Kaffe *et al*. 2012 (Kaffe et al., 2012)	Healthy	18-60	40	Randomized, parallel	12 h, 24 h and 168 h (7 days)	2 g plant sterols without food vector	?		↘ vitamin D 3 (12 h and 24 h after administration) ↘ 25-hydroxyvitamin D 3 (at 168 h)
Sialvera *et al*. 2013 (Sialvera et al., 2013)	Metabolic syndrome	30-65 (48 ±11/Test and 45 ±11/control)	108	Randomized, parallel arm, placebo-controlled design	2 months	Yogurt beverage (4 g of phytosterols)	Habitual westernized diet		No change for vitamin A and □-tocopherol
Petrogianni *et al*. 2014 (Petrogianni et al., 2014)	Hypercholol.	40-60	108 (53/test + 55/control)	Randomized, parallel	3 months	Phytosterol enriched low-fat milk (2.5 g)	Apparently regular		No change for □-carotene

Normochol, normocholesterolemic; Hyperchol, hypercholesterolemic ; NS, Non Significant; ↗, Significant increase ; ↘, significant decrease.

Table 2. Median, min. and max. values of percent changes of fat-soluble vitamins and their precursors as extracted from 38 human interventional studies[1].

Vit. A (retinol)	Vit. D	Vit. E	Vit. K (phylloquinone)	□-tocopherol	□-tocopherol	□-tocopherol	□-tocopherol	□+□-tocopherols	□-carotene	□-carotene	□+□-carotene	□-cryptoxanthin
Min./max. values (median values) for the percent changes in non-standardized serum and plasma concentrations (%)												
NS/+9	-4/+24 (+14)	NS	-14/NS	-16/NS (-10)	-9	-12/NS (-12)	-10/NS	-13/NS	-42/NS (-13)	-74/+17 (-24)	-43/-8 (-21)	-32/NS (-16%)
Number of studies[2] for significant fat-soluble vitamins reduction (↘) and increase (↗), and for no significant effect (non-standardized results)												
21NS/1↗	1↘/11NS/3↗	2NS	1↘/7NS	16↘/14NS	1↘	2↘, 9NS	1↘/4NS	3NS	9↘/13NS	20↘/13NS/1↗	2↘	5↘/9NS
Min./max. values (median values) for the percent changes in standardized serum and plasma concentrations (%)												
-16/+10 (+9)	-[3]	-	NS	NS/+8 (+6)	-	NS	NS/+25	NS/+25	-33/NS (-18)	-37/NS (-25)	-43/NS (-20)	-9/NS (-9)
Number of studies[2] for significant fat-soluble vitamins reduction (↘) and increase (↗), and for no significant effect (standardized results)[4]												
1↘/3NS/2↗	-	-	1NS	16NS/2↗	-	6NS	3NS/1↗	2NS/1↗	6↘, 14NS	8↘/15NS	2↘/1NS	2↘/9NS

[1]Among the 52 intervention studies, bioavailability studies and studies including foods enriched with fat-soluble vitamins and/or their precursors, and studies including diets enriched with fruits and vegetables were excluded from calculations, i.e., n = 14. Percent changes were calculated from the differences in plasma fat-soluble and carotenoid before and after the intervention with phytosterols/stanols-enriched food vectors (as indicated in Table 1); [2]As can be seen from Table 1, number of studies differ according to whether or not results are standardized; [3]No data

available; [4]Standardization is based on either total, triglycerides or LDL cholesterol concentrations; NS, not significant.

