Review

Consumption of plant sterol-enriched foods and effects on plasma plant sterol concentrations — A meta-analysis of randomized controlled studies

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ABSTRACT

Objective: Intake of plant sterol (PS)-enriched foods effectively lowers plasma total- and LDL-cholesterol concentrations while increasing plasma PS concentrations. The magnitude of this increase has not been systematically assessed. This study aimed to investigate the effect of PS-enriched foods on plasma PS concentrations by performing a meta-analysis of randomized controlled studies.
Methods: Published PS intervention studies reporting plasma PS concentrations were searched through June 2012. Studies were selected that fulfilled pre-defined in- and exclusion criteria. Data were extracted, particularly on campesterol, sitosterol, total- and LDL-cholesterol. Random-effects models were used to calculate net effects while weighing each study by the inverse of its variance. Potential sources of heterogeneity were investigated.
Results: The meta-analysis included data from 41 studies (55 strata) with in total 2084 subjects. The average dose of PS from enriched foods was 1.6 g/d (range: 0.3–3.2 g/d). Plasma sitosterol and campesterol concentrations were increased by on average 2.24 μmol/L (31%) and 5.00 μmol/L (37%), respectively, compared to control. Total- and LDL-cholesterol were reduced by on average 0.36 mmol/L (5.9%) and 0.33 mmol/L (8.5%), respectively. The increase in sitosterol and campesterol was impacted by the dose of PS, the baseline PS concentration and the PS composition of the test products. In the highest PS dose category (2.0–3.2 g/d), increases in sitosterol and campesterol were on average 3.56 and 7.64 μmol/L, respectively.
Conclusion: Intake of PS-enriched foods increases plasma sitosterol and campesterol concentrations. However, total PS remain below 1% of total sterols circulating in the blood.
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1. Introduction

Plant sterols (PS) are found in all foods of plant origin and are structurally similar to cholesterol except for a slight difference in their side chain, i.e. an additional ethyl or methyl group at C24. The two major PS are sitosterol (24α-ethylcholesterol) and campesterol (24α-methylcholesterol). Intake of PS-enriched foods or supplements has been shown to effectively lower total cholesterol (TC) and low-density lipoprotein (LDL)-cholesterol concentrations [1,2]. Based on recent meta-analyses, a PS intake of 2 g/d lowers LDL-cholesterol by on average 0.31–0.34 mmol/L or 8–10% [3–5]. Elevated TC, and especially LDL-cholesterol, is an established risk factor for cardiovascular disease (CVD) and reducing cholesterol by dietary or drug interventions is known to reduce the risk of CVD [6,7]. Hence, the cholesterol-lowering properties of PS have been acknowledged by health associations such as the National Cholesterol Education Program Adult Treatment Panel III [8], the American Heart Association [9], the European Society of Cardiology and the European Atherosclerosis Society [10].

PS lower plasma cholesterol by partly inhibiting cholesterol absorption in the gut, mainly through competition with cholesterol for micellar incorporation [11]. In contrast to cholesterol, PS themselves are not bioavailable in significant quantities as they are excreted back from the intestinal mucosa into the intestinal lumen by the heterodimer ATP-binding cassette (ABC) transporters ABCG5/8 [12]. Only a small amount of dietary PS can be absorbed and reaches the systemic circulation [13]. Furthermore, PS are not synthesized in the human body. As such, circulating PS concentrations are ∼200 times lower compared to cholesterol concentrations in subjects consuming habitual diets [14].

When people consume the recommended dose of 2 g/d PS for cholesterol-lowering purposes, they ingest 7–10 times more PS than what is normally reached when consuming typical Western diets which contain natural sources of PS such as vegetable oils, cereals, vegetables, fruits, nuts and seeds. In these Western-type diets, PS intakes range between 200 and 300 mg/d [15–18] whilst vegetarians can consume up to 500–1000 mg/d of PS [19,20]. Despite the low bioavailability of PS, higher intakes of PS, especially with enriched foods, do eventually result in increased plasma/serum PS concentrations.

Recently, potential health concerns have been voiced related to elevated PS concentrations following the intake of PS-enriched foods mainly because of two reasons. First, patients with homozygous sitosterolemia, a rare genetic disorder with mutations in *ABCG5/8* genes, have extremely elevated PS concentrations (∼500–1200 μmol/L) and often experience early onset of atherosclerosis independent of circulating cholesterol [21,22]. Second, some, but not all, observational studies suggest a positive association between modestly elevated PS concentrations and CVD risk although the overall evidence, as summarized in a recent meta-analysis, does not support such an association [23].

Until now, the effect of PS-enriched food intake on plasma PS concentrations has not yet been systematically investigated and the size of the increase in circulating PS seems often overestimated by referring to single studies. Therefore, the objective of this study was to perform a meta-analysis of randomized controlled studies to estimate the absolute and relative change in plasma concentrations of the main PS (i.e. sitosterol and campesterol) with and without correction for TC concentrations after consumption of PS-enriched food. Additionally, we estimated the change in plasma LDL-cholesterol and TC concentrations in the selected PS intervention studies. Furthermore, sources that could possibly explain some of the between-study heterogeneity in changes in plasma PS and cholesterol concentrations were investigated.

2. Methods

2.1. Search strategy

To retrieve as many potentially relevant studies as possible, six databases (Medline, Embase, Cab Abstracts, Food Science & Technology Abstracts, HCA Plus and Biosis) were systematically searched through June 2012. For this, a search strategy was developed including the Medical Subject Heading 'phytosterols' and the search terms 'plant sterol* or phytosterol* or sitosterol* or campesterol* or stigmasterol* or brassicasterol*' and 'blood* or plasma or serum', limited to humans and intervention studies were possible. There was no restriction on language. For simplicity, throughout this paper, the term "plasma" is used when referring to plasma or serum depending on what has been used in the different studies.

2.2. Selection of studies

The following criteria for selecting eligible studies were predefined: (a) randomized placebo-controlled study in humans (studies with (familial hypercholesterolemic) children were allowed); (b) oral intake of PS-enriched foods or supplements as active treatment (throughout this paper, the term "enriched foods" encompasses also supplements which were used in only a few studies); (c) absence of co-intervention from which consumption of PS-enriched foods could not be isolated; (d) no studies with colectomized patients or patients with hetero- or homozygous sitosterolemia; (e) duration of treatment of at least two weeks; (f) reporting of treatment effects on plasma sitosterol and campesterol concentrations; (g) treatment with "common" PS defined as 4-desmethylsterols extracted from common vegetable oils and no ferulated PS such as from rice bran oil and/or sheanut oil; (h) dose of PS < 10 g/d; (i) composition of the phytosterol mixture containing at least 80% PS (max 20% plant stanols); (j) no treatment with ezetimibe; and (k) no conference proceedings or duplicates.

Selection of studies was done in two rounds. In the first selection round, titles and abstracts were screened and those studies that were obviously not fulfilling the pre-defined selection criteria were excluded, e.g. reviews, studies testing other ingredients than PS or acute-effect studies. Because investigating effects on plasma PS is usually not the primary objective in PS intervention studies, we did not limit our search by only selecting studies that reported results on plasma PS concentrations in their abstracts. In the second selection round, full publications were read to judge eligibility of the studies. A co-intervention was defined as any additional test ingredient next to PS which was not added to the placebo intervention (e.g. the portfolio diet containing soluble fiber, nuts, PS and soy protein vs. a placebo diet). The source of fatty acid esters used to esterify PS into PS esters was not considered as a co-intervention. The PS mixtures used in the studies were not allowed to contain more than 20% plant stanols [24] as stanols are known to reduce plasma PS concentrations [25]. Studies including ezetimibe treatment were not selected because ezetimibe is known to directly impact plasma PS concentrations via mechanisms in the gut. In case of indecisiveness, eligibility was discussed amongst authors until consensus was reached.

2.3. Data extraction and transformation

Data were collected on (a) publication characteristics (reference details and year of publication); (b) study characteristics (parallel or crossover, sample size and study duration); (c) subject characteristics (health status of subjects, mean age, mean BMI and gender distribution); (d) treatment characteristics (PS dose, form of PS (free or esterified PS), food format, PS source, etc); (e) measurement characteristics (methodology used and serum or plasma); and (f) outcome variables (plasma concentrations of sitosterol, campesterol (including those standardized for TC, e.g. expressed in μmol/mmol TC), LDL-cholesterol, TC and high-density lipoprotein (HDL-)cholesterol). We have not assessed the quality of the individual studies because scoring of quality is rather subjective and excluding studies based on this subjective scoring was judged not appropriate.

For each of the outcome variables, data (mean absolute concentration and accompanying variance measure) were extracted at baseline and at end-of-intervention. When outcome variables were measured at different time points, the data closest to 4 weeks of intervention were selected in order to standardize the intervention duration amongst the studies. Original authors were contacted in case the sitosterol and campesterol data were solely expressed as concentrations corrected for TC [26–28].

In case concentrations of cholesterol were expressed in mg/dL, data were transformed to derive concentrations in mmol/L by using the molecular weight of cholesterol (386.65 g/mol). In case concentrations of sitosterol and campesterol were expressed in mg/L, mg/dL, μg/dL, ng/dL, μg/mL or ng/mL, data were transformed based on the molecular weights of sitosterol (414.71 g/mol) or campesterol (400.68 g/mol) to derive concentrations in μmol/L. These transformations were done both for means and SEs or SDs.

Control-adjusted absolute (μmol/L or mmol/L) and relative (%) changes plus accompanying within-study SEs for sitosterol, campesterol, LDL-cholesterol, TC and HDL-cholesterol were calculated for each study. For parallel studies, the absolute and relative changes plus accompanying SEs were calculated based on the average concentrations and variance measures at baseline and at end-of-intervention of treatment and control groups. For crossover studies that reported baseline data, the absolute and relative changes were calculated similarly as for the parallel studies. Otherwise, these were calculated based on the data at the end of the treatment and control periods. In Supplemental Appendix 1, a complete overview is provided of the formulas that were used to transform the data.

2.4. Statistical analysis

For each of the main outcome variables, a net effect was calculated according to a random-effects model while weighing the studies by the inverse of their within-study variance ($1/SE^2$) [29]. This was done for baseline concentrations, end-of-intervention concentrations, absolute changes and relative changes. In contrast to fixed-effects models, random-effects models take into account both the within-study variation as well as the large variation between studies and assume that the treatment effects of the individual studies vary around some overall average treatment effect.

Funnel plots were developed with the effect sizes of all individual studies expressed against their precisions (1/SE). These plots visualize the likeliness of heterogeneity (when effect sizes fall outside the confidence limits) as well as the likeliness of publication bias (when clear holes in the funnel (i.e. asymmetry) are detected). Heterogeneity was furthermore assessed by calculating Q-statistics and I^2-statistics [29] whereas publication bias was analyzed according to Egger tests [30]. Forest plots were developed for the absolute (μmol/L) and relative changes (%) in sitosterol and campesterol.

Covariate analysis was performed to investigate the impact of pre-specified covariates on the absolute and relative changes in plasma PS and cholesterol concentrations after consumption of PS-enriched foods. These covariates were dose of PS tested, baseline PS or cholesterol concentration and PS composition (i.e. amount of sitosterol or campesterol in the PS mixture of the test products). Subgroup analysis was performed for determining differences between subgroups after stratification based on the above mentioned covariates. Also meta-regression analysis was performed for assessing their correlations with the effect sizes found.

P-values below 0.05 were considered statistically significant based on two-sided hypothesis testing. All analyses were performed with the statistical software package SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA). The PRISMA statement guidelines for reporting in systematic reviews and meta-analyses were followed.

3. Results

3.1. Overview of included studies

With the systematic search, 1034 papers were identified. After two selection rounds, 41 human intervention studies including 55 strata were judged eligible for inclusion in the current meta-analysis (Fig. 1). Most of the studies were excluded because they were no randomized controlled studies with human subjects, investigated a different active ingredient or did not report plasma or serum PS concentrations.

Of the 41 studies, 21 studies were parallel studies [26–28,31–48], 19 were crossover studies [49–67] and 1 paper described a parallel and a crossover study [68]. Non-standardized concentrations of sitosterol and campesterol were available for all studies (55 strata). For 12 studies (14 strata), PS concentrations corrected for TC concentrations (e.g. expressed in μmol/mmol TC) were available.

In total, 2084 subjects were included in the studies. The average age of the subjects was 49.3 years (range: 10.5–60.1 years) and the average BMI was 26.0 kg/m^2 (range: 19.0–35.2 kg/m^2). Six strata included healthy or normocholesterolemic subjects, 39 strata included hypercholesterolemic but otherwise healthy subjects and 10 strata included (hypercholesterolemic) subjects characterized as metabolic syndrome patients, diabetics or statin users.

The median duration of studies was 28 days (range: 21–315 days). The PS dose tested was an average 1.6 g/d (median: 1.7 g/d; range: 0.3–3.2 g/d). The majority of studies used (low-fat) spreads (*n* = 28) whilst others used dairy products like yoghurt, milk etc
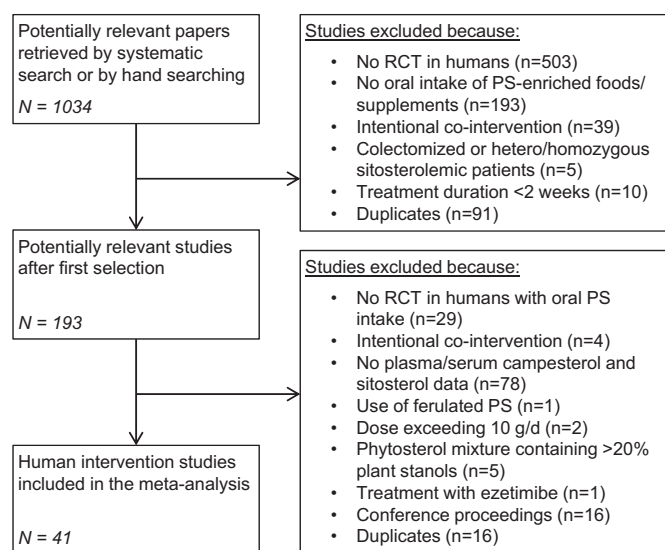
Fig. 1. Flow-chart of the study selection process.

($n = 12$) or other formats like dressing, mayonnaise, bread or supplements ($n = 15$). PS were in most cases esterified to different fatty acids ($n = 43$); only 12 strata used free PS as active ingredient. Depending on the source of PS, PS mixtures typically contained 20—30% campesterol, 45—50% sitosterol and some other minor sterols/stanols for soybean PS or 5—10% campesterol, 75—80% sitosterol and some other minor sterols/stanols for tall oil PS [33]. Tables 1 and 2 show overviews of the parallel and crossover studies, respectively, including sitosterol and campesterol data. In Supplemental Appendix 2, an overview is provided summarizing the blood cholesterol data.

3.2. Plasma PS outcomes

At baseline, plasma sitosterol and campesterol concentrations were on average 6.92 and 13.07 µmol/L, respectively. After intake of on average 1.6 g/d PS from enriched foods, plasma PS concentrations were significantly increased by on average 2.24 µmol/L for sitosterol and 5.00 µmol/L for campesterol, compared to control. Corresponding relative increases were 31.3% and 37.3%, respectively. Total PS remained below 1% of total sterols circulating in the

Table 1
Overview of parallel studies.

Reference information	Subject characteristics					Treatment characteristics				Plasma plant sterols			
	Sample size		Gender (% male)	Age (y)	BMI (kg/m²)	Free or ester	Dose (g/d)[a]	Food format	Duration (days)	Sitosterol		Campesterol	
	Control	Active								Absolute change (µmol/L)	Relative change (%)	Absolute change (µmol/L)	Relative change (%)
Carr et al., 2009	16	16	50.0	37.1	25.5	Ester	1.8[a]	Capsules	28	0.97	16.3	3.69	42.5
Christiansen et al., 2001 stratum 1[b]	46	46	35.5	50.7	25.7	Free	1.5	Rapeseed oil margarine	90	0.97	33.0	−1.09	−12.3
Christiansen et al., 2001 stratum 2[b]	46	42	35.5	50.7	25.9	Free	3.0	Rapeseed oil margarine	90	1.02	36.6	−1.52	−21.2
Clifton et al., 2008 stratum 1[b]	39	37	56.6	55.2	26.8	Ester	1.6	Low-fat margarine	21	1.70	23.7	6.63	67.6
Clifton et al., 2008 stratum 2[b]	39	39	48.7	54.5	26.9	Ester	1.6	Low-fat margarine	21	6.36	78.9	1.37	11.4
Clifton et al., 2008 stratum 3[b]	39	36	56.0	54.2	26.7	Ester	1.6	Low-fat margarine	21	3.41	46.0	5.87	49.3
Hansel et al., 2007	99	95	67.0	48.9	23.6	Ester	1.6	Low-fat fermented milk	42	1.81	27.3	−0.75	−14.1
Hendriks et al. 2003[b,c]	96	89	48.6	48.0	24.9	Ester	1.6	Low-fat margarine	182	2.67	23.7	13.38	76.2
Hernandez-Mijares et al. 2010[b,d]	24	31	27.3	49.5	28.2	Ester	2.0	Low-fat milk	90	8.53	93.4	6.64	105.3
De Jong et al. 2006[e]	11	15	46.2	58.1	26.8	Ester	2.5	Low-fat margarine	112	4.10	36.4	25.50	95.3
De Jong et al. 2008[e]	17	18	60.0	59.5	25.7	Ester	2.5	Low-fat margarine	315	3.84	35.2	16.52	96.4
Korpela et al., 2006	82	82	21.3	57.3	27.0	Free	1.9[f]	Low-fat milk products	42	3.62	51.7	−1.25	−6.5
Kurokawa et al., 2008	27	32	69.9	44.7	23.2	Ester	0.8	Dressing	84	2.41	50.0	4.99	40.0
Maki et al., 2001 stratum 1[b,c]	92	92	44.0	58.1	27.5	Ester	1.1	Low-fat margarine	35	1.11	19.8	4.49	24.1
Maki et al., 2001 stratum 2[b,c]	92	40	42.4	58.4	27.3	Ester	2.2	Low-fat margarine	35	2.36	34.4	9.53	43.6
Mannarino et al., 2008	56	60	44.0	50.1	25.0	Ester	1.6	Low-fat fermented milk	42	0.58	3.9	−0.50	−3.5
Masuda et al., 2007	49	48	59.8	46.7	23.1	Ester	0.8	Drink	84	0.30	5.0	−0.07	−1.7
Neil et al. 2001[b,c,d]	29	29	41.9	51.6	26.0	Ester	2.5	Margarine	56	1.17	20.4	7.21	71.5
Nittynen et al., 2007 study 2	14	12	57.7	45.9	26.0	Free	2.0	Low-fat yogurt drink	56	9.65	56.9	6.99	29.6
Plana et al. 2008[c,e]	40	43	41.0	51.4	26.7	Ester	1.6	Low-fat fermented milk	42	2.52	33.5	0.79	16.7
Saito et al., 2006 stratum 1	17	16	100.0	38.4	23.6	Ester	0.3	DAG-containing mayonnaise	28	0.42	5.5	1.60	9.4
Saito et al., 2006 stratum 2	17	16	100.0	39.2	23.6	Ester	0.4	DAG-containing mayonnaise	28	1.79	23.9	4.60	27.1
Saito et al., 2006 stratum 3	17	17	100.0	38.7	23.9	Ester	0.5	DAG-containing mayonnaise	28	0.93	10.5	3.80	19.8
Seki et al., 2003a	28	32	100.0	39.1	24.2	Ester	0.5	Vegetable oil-based bread	28	0.72	16.1	2.25	39.5
Seki et al., 2003b	11	11	100.0	41.2	24.2	Ester	1.3	Vegetable oil-based bread	28	0.96	23.2	3.74	78.4
Takeshita et al., 2007a	21	18	100.0	37.0	24.4	Ester[g]	0.4	DAG-containing mayonnaise	28	1.01	17.4	3.62	27.1
Takeshita et al., 2008	15	14	20.7	59.4	21.9	Free	0.5	DAG-containing cooking oil	84	−0.46	−5.0	1.85	10.2
Varady et al., 2004 study 1	20	18	31.6	56.6	26.3	Ester	1.8	Low-fat margarine	56	0.95	28.9	2.94	38.4
Varady et al., 2004 study 2	18	18	22.2	52.1	29.9	Ester	1.8	Low-fat margarine	56	1.04	24.0	3.99	48.9

[a] PS dose expressed as free equivalents. Carr et al., 2009 reported the PS dose as esters; the amount of free equivalents was calculated assuming that 40% of PS esters are fatty acids and 60% are PS.

[b] The following papers reported serum/plasma PS concentrations in a subset of the total number of subjects included in the study: Christiansen et al. ($n = 52$), Clifton et al. ($n = 25$), Hendriks et al. ($n = 83$), Hernandez-Mijares et al. ($n = 34$), Neil et al. ($n = 54$) and Maki et al. ($n = 71$).

[c] Cholesterol and plasma PS data are based on raw data.

[d] No average age was reported; thus, the average of the medians per group was used.

[e] Non-standardized plasma PS data were obtained from original authors.

[f] The dose of PS was calculated by taking the average of 2, 2 and 1.65 g/d which were the doses used in the different foods.

[g] PS were mostly esterified PS.

Table 2
Overview of crossover studies.

Reference information	Subject characteristics				Treatment characteristics				Plasma plant sterols			
	Sample size	Gender (% male)	Age (y)	BMI (kg/m²)	Free or ester	Dose (g/d)[a]	Food format	Duration (days)	Sitosterol		Campesterol	
									Absolute change (μmol/L)	Relative change (%)	Absolute change (μmol/L)	Relative change (%)
AbumWeis et al., 2006 stratum 1	30	–	59.0	28.0	Free	1.7	Margarine	29	2.90	41.4	0.80	4.3
AbumWeis et al., 2006 stratum 2	30	–	59.0	28.0	Ester	1.7	Margarine	29	1.70	24.3	5.50	29.6
Amundsen et al. 2004[b]	41	46.3	10.5	19.0	Ester	1.6	Low-fat margarine	56	4.00	33.4	10.37	76.3
Casas-Agustench et al., 2012	43	51.2	49.0	26.6	Ester	2.0	Skimmed milk	28	12.01	90.6	9.22	62.8
Clifton et al., 2004 stratum 1[c]	58	39.7	54.0	26.2	Ester	1.6	Bread	21	2.70	31.6	4.09	44.1
Clifton et al., 2004 stratum 2[c]	58	39.7	54.0	26.2	Ester	1.6	Milk	21	2.34	27.4	4.89	52.7
Demonty et al., 2006	21	52.4	54.2	25.9	Ester	1.7	Orange juice	29	1.38	19.9	8.19	67.9
Hallikainen et al., 2000	34	47.6[d]	48.8	24.9	Ester	2.0	Margarine	28	2.00	24.4	6.89	35.8
Heggen et al., 2010 stratum 1	59	72.9	52.0	24.8	ester	2.0	Low-fat margarine	28	5.73	83.1	1.10	4.8
Heggen et al., 2010 stratum 2	59	72.9	52.0	24.8	Ester	2.0	Low-fat margarine	28	5.97	86.5	15.05	65.6
Houweling et al., 2009 study 1	41	100.0	50.4	27.8	Ester	2.0	Low-fat margarine	28	2.77	23.1	4.25	21.0
Houweling et al., 2009 study 2	41	100.0	52.1	29.0	Ester	2.0	Low-fat margarine	28	2.71	34.7	3.23	27.0
Jakulj et al., 2005	39	87.5	55.5	25.9	Ester	2.0	Low-fat margarine	28	1.91	20.7	10.96	78.7
Jones et al., 2000	15	100.0	–	–	Ester	1.8	Low-fat margarine	21	2.50	29.1	8.60	67.3
Kratz et al., 2007	10	–	30.0	21.9	Ester	2.0	Low-fat margarine	42	0.90	12.5	4.00	24.0
Lau et al., 2005 study 1[b]	14	35.7	54.5	30.2	Free	1.8	Margarine	21	0.84	34.1	0.31	8.6
Lau et al., 2005 study 2[b]	15	40.0	55.1	26.9	Free	1.8	Margarine	21	0.66	20.3	0.72	9.3
Mussner et al., 2002	62	38.7	42.0	24.0	Ester	1.8	Margarine	21	1.57	35.3	6.96	78.2
Myrie et al., 2012	15	53.3	33.8	30.4	Free	1.6	Capsules	29	0.21	3.0	2.84	22.8
Nittynen et al., 2007 study 1	15	100.0	41.0	26.0	Free	1.0	Low-fat yogurt drink	28	2.41	38.5	5.99	34.3
Ooi et al., 2007	9	100.0	60.1	35.2	Ester	2.0	Breakfast cereal and margarine	28	1.95	44.3	5.19	80.6
Rudkowska et al., 2008 stratum 1	26	–	59.6	26.4	Ester	1.6	Low-fat yogurt	30	0.85	42.7	2.74	27.8
Rudkowska et al., 2008 stratum 2	26	–	59.6	26.4	Ester	1.6	Low-fat yogurt	30	1.02	55.5	3.87	39.0
Takeshita et al., 2007b	14	0.0	52.2	23.0	Free	0.6	DAG-containing cooking oil	28	1.44	22.2	4.38	33.3
Vanstone et al., 2002	15	60.0	47.8	30.8	Free	1.8	Butter	21	2.40	28.3	13.10	90.9
Weststrate et al. 1998[e]	40	50.0	46.2	24.2	Ester[f]	3.2	Margarine	21	2.89	37.5	12.23	71.0

[a] PS dose expressed as free equivalents.
[b] The following papers reported serum PS concentrations in a subset of the total number of subjects included in the study: Amundsen et al. ($n = 29$) and Lau et al. ($n = 27$).
[c] Incomplete crossover design: not all subjects received all treatments ($n_{control} = 58$, $n_{bread} = 36$, $n_{milk} = 40$).
[d] Gender distribution is based on 42 subjects (8 dropped out).
[e] Age, gender, cholesterol and plasma PS data are based on raw data of $n = 40$. BMI is based on total n in the study ($n = 100$).
[f] PS were mostly esterified PS.

blood. When corrected for TC, sitosterol concentrations significantly increased by on average 0.59 μmol/mmol TC (41.7%) and campesterol by on average 1.34 μmol/mmol TC (60.8%). Table 3 gives an overview of the weighed net effects. Forest plots of the absolute changes in sitosterol and campesterol are shown in Fig. 2. Forest plots of the relative changes are provided in Supplemental Appendix 3.

For both absolute and relative changes in sitosterol and campesterol concentrations, there was significant heterogeneity between the studies as assessed by inspecting funnel plots (a considerable number of studies reported effects outside the confidence limits) and calculating I^2-statistics (P-value<0.05) (Supplemental Appendix 4). Furthermore, regression analysis of the standard normal deviate as a function of the precision and the

Table 3
Weighed net effects (baseline, end-of-intervention, absolute change and relative change) of plasma sitosterol, campesterol, LDL-cholesterol, total cholesterol and HDL-cholesterol, based on random effects models.

Parameter	Unit	Baseline concentration[a]	Concentration after PS intervention[b]	Absolute change vs. placebo	Relative change vs. placebo (%)
β-Sitosterol	μmol/L	6.92 (6.23; 7.61)	9.29 (8.20; 10.38)	2.24 (1.71; 2.77)	31.3 (26.0; 36.7)
	μmol/mmol TC[c]	1.22 (0.88; 1.56)	1.77 (1.14; 2.41)	0.59 (0.25; 0.92)	41.7 (31.0; 52.5)
Campesterol	μmol/L	13.07 (11.65; 14.48)	18.18 (15.99; 20.38)	5.00 (3.86; 6.14)	37.3 (29.3; 45.3)
	μmol/mmol TC[c]	2.10 (1.63; 2.56)	3.39 (2.43; 4.34)	1.34 (0.83; 1.85)	60.8 (44.7; 76.9)
LDL-cholesterol	mmol/L	3.90 (3.76; 4.03)	3.59 (3.47; 3.72)	−0.33 (−0.37; −0.30)	−8.5 (−9.2; −7.7)
Total cholesterol	mmol/L	6.04 (5.90; 6.18)	5.69 (5.56; 5.82)	−0.36 (−0.40; −0.32)	−5.9 (−6.5; −5.3)
HDL-cholesterol	mmol/L	1.42 (1.37; 1.47)	1.41 (1.36; 1.47)	−0.00 (−0.02; 0.01)	−0.1 (−1.1; 0.9)

Expressed as means (95%CI).
[a] The weighed average baseline concentration was calculated based on the baseline concentrations in the active and placebo groups for parallel studies. For crossover studies, the baseline concentrations were used when reported; otherwise the end-of-intervention concentrations of the placebo periods were used.
[b] The weighed average concentration after PS intervention was calculated based on the concentrations after PS intervention in the active treatment groups for parallel studies, and based on the end-of-intervention concentrations of the active periods in case of crossover studies.
[c] The weighted net effects of the PS to cholesterol ratios were based on only 12 studies (14 strata) that reported plasma PS concentrations corrected for total cholesterol concentrations. The non-standardized weighed net effects are based on 41 studies (55 strata).
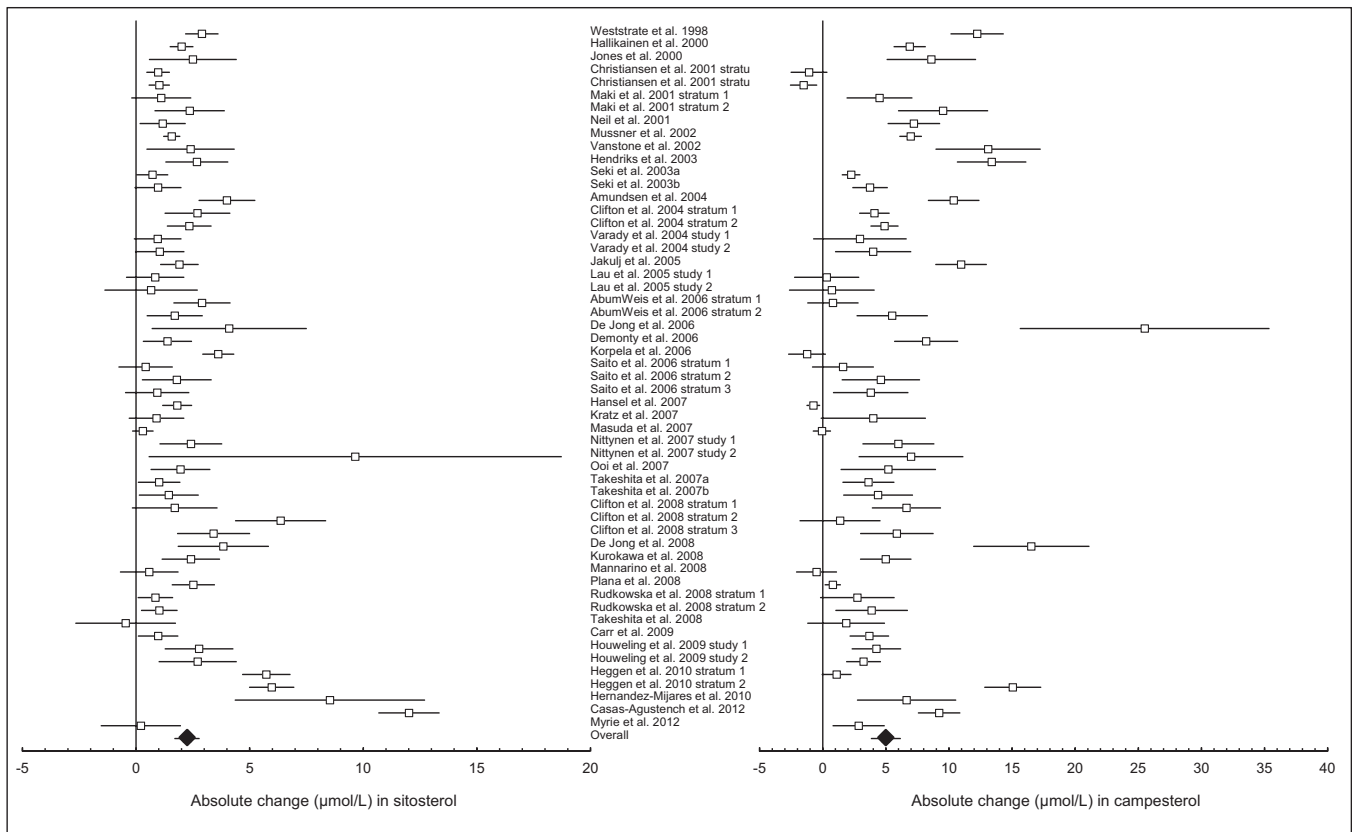
Fig. 2. Forest plots of the absolute (μmol/L) changes in plasma sitosterol and campesterol concentrations.

asymmetrical shape of the funnel plots indicated that publication bias was likely present in all sitosterol and campesterol analyses (Egger test: P-value (intercept) < 0.05; studies reporting relatively small increases in plasma PS concentrations at the bottom of the funnel seemed lacking).

3.3. Plasma cholesterol outcomes

LDL-cholesterol and TC concentrations at baseline were on average 3.90 and 6.04 mmol/L, respectively. LDL-cholesterol was reduced by on average 0.33 mmol/L (8.5%) and TC by 0.36 mmol/L (5.9%) with an average PS intake of 1.6 g/d, compared to control (Table 3). Heterogeneity was statistically significant for absolute changes in TC (P-value = 0.029) whereas it was not significant for absolute and relative changes in LDL-cholesterol and for relative changes in TC (P-value >0.05). The heterogeneity in cholesterol changes was clearly less obvious as compared to the heterogeneity in plasma PS changes. Visual inspection of symmetrical funnel plots as well as the outcomes of the Egger tests (P-value of intercept ranging between 0.397 and 0.613) suggested absence of publication bias for LDL-cholesterol and TC (Supplemental Appendix 5). HDL-cholesterol did not change upon PS intervention (−0.00 mmol/L or −0.1%; Table 3).

3.4. Covariate analyses

Meta-regression analyses revealed that dose of PS, baseline PS concentration and PS composition significantly impacted the absolute changes in plasma PS concentrations. Absolute increases in sitosterol and campesterol were larger in studies with higher doses of PS ($\beta = 1.02$, P-value = 0.014 and $\beta = 2.37$, P-value = 0.009,

respectively), with higher average baseline concentrations ($\beta = 0.39$, P-value <0.001 and $\beta = 0.35$, P-value <0.001, respectively), and with higher amount of either sitosterol or campesterol in the PS mixture ($\beta = 0.06$, P-value = 0.004 and $\beta = 0.27$, P-value = <0.001, respectively). When looking at the relative changes, the impact of baseline concentrations was, as expected, not present anymore. Subgroup analyses showed comparable results except for a weaker (non-significant) impact of PS composition on absolute and relative changes in plasma sitosterol and a weaker impact of PS dose on relative changes in plasma campesterol. In the subgroup with the highest-dose studies (2.0–3.2 g/d PS), increases in sitosterol and campesterol were on average 3.56 μmol/L (42.2%) and 7.64 μmol/L (47.9%), respectively. The results of the covariate analyses are shown in Table 4.

3.5. Post hoc analyses

To investigate the shape of the dose–response relationship between PS doses and changes in plasma PS, we established continuous dose–response curves based on first-order elimination functions. A slight tapering-off effect seemed present for changes in both plasma sitosterol (Fig. 3, Panel A) and campesterol concentrations (Fig. 3, Panel B). It should however be noted that these curves are severely limited by the heterogeneous distribution of the observed changes across the range of doses included.

In addition, we investigated the impact of food format (dairy-type foods vs. (low-fat) margarine), blood matrix (serum vs. plasma), subjects' health status (diabetics/metabolic syndrome patients vs. hypercholesteromic subjects vs. normocholesterolemic/healthy subjects) and study duration (≤4 weeks vs. >4 weeks) on the changes in plasma PS concentrations. No significant

Table 4
Results of the covariate analysis for absolute and relative changes in plasma sitosterol, campesterol, LDL-cholesterol and total cholesterol concentrations.

Trial characteristic	Stratification variable	No of study arms	Subgroup analysis			Meta-regression analysis		Subgroup analysis			Meta-regression analysis	
			Change vs. placebo	95% CI	P-value between subgroups[a]	β	P-value meta-regression[b]	Change vs. placebo	95% CI	P-value between subgroups[a]	β	P-value meta-regression[b]
			Absolute change (μmol/L) in sitosterol					Relative change (%) in sitosterol				
Baseline concentration	Below median (≤6.9 μmol/L)	26	1.38	(0.71; 2.05)	<0.001	0.39	<0.001	28.1	(20.3; 36.0)	0.284	0.4	0.677
	Above median (>6.9 μmol/L)	29	3.08	(2.41; 3.76)				33.9	(26.8; 41.1)			
Dose of PS	≥0.3 g/d and ≤1.5 g/d	13	1.08	(0.13; 2.04)	0.001	1.02	0.014	18.7	(9.0; 28.5)	0.002	12.0	0.002
	>1.5 g/d and <2.0 g/d	25	2.00	(1.31; 2.69)				30.0	(22.9; 37.2)			
	≥2.0 g/d and ≤3.2 g/d	17	3.56	(2.68; 4.44)				42.2	(33.6; 50.9)			
PS composition (% sitosterol)	Below median (<50%)	27	1.81	(1.06; 2.56)	0.128	0.06	0.004	26.8	(19.1; 34.4)	0.107	0.8	<0.001
	Above median (≥50%)	28	2.62	(1.90; 3.34)				35.4	(28.2; 42.6)			
			Absolute change (μmol/L) in campesterol					Relative change (%) in campesterol				
Baseline concentration	Below median (≤12.6 μmol/L)	28	3.44	(1.97; 4.91)	0.003	0.35	<0.001	35.8	(24.3; 47.4)	0.724	0.4	0.572
	Above median (>12.6 μmol/L)	27	6.64	(5.10; 8.17)				38.7	(27.6; 49.8)			
Dose of PS	≥0.3 g/d and ≤1.5 g/d	13	3.03	(0.92; 5.15)	0.003	2.37	0.009	24.1	(8.7; 39.5)	0.084	12.8	0.042
	>1.5 g/d and <2.0 g/d	25	4.26	(2.72; 5.79)				37.4	(26.0; 48.9)			
	≥2.0 g/d and ≤3.2 g/d	17	7.64	(5.72; 9.55)				47.9	(33.8; 62.0)			
PS composition (% campesterol)	Below median (<25%)	27	3.11	(1.67; 4.54)	<0.001	0.27	<0.001	26.4	(15.9; 37.0)	0.005	1.6	0.001
	Above median (≥25%)	28	6.85	(5.39; 8.31)				47.5	(37.1; 58.0)			
			Absolute change (mmol/L) in LDL-cholesterol					Relative change (%) in LDL-cholesterol				
Baseline concentration	Below median (≤3.9 mmol/L)	26	−0.26	(−0.31; −0.21)	0.001	−0.10	0.011	−7.4	(−8.7; −6.0)	0.057	−0.6	0.559
	Above median (>3.9 mmol/L)	29	−0.37	(−0.41; −0.34)				−8.9	(−9.8; −8.1)			
Dose of PS	≥0.3 g/d and ≤1.5 g/d	13	−0.25	(−0.32; −0.18)	0.038	−0.07	0.017	−6.6	(−8.3; −4.8)	0.052	−1.7	0.015
	>1.5 g/d and <2.0 g/d	25	−0.35	(−0.40; −0.30)				−8.8	(−9.8; −7.7)			
	≥2.0 g/d and ≤3.2 g/d	17	−0.35	(−0.40; −0.31)				−9.1	(−10.2; −7.9)			
			Absolute change (mmol/L) in total cholesterol					Relative change (%) in total cholesterol				
Baseline concentration	Below median (≤6.0 mmol/L)	26	−0.26	(−0.32; −0.20)	<0.001	−0.14	<0.001	−4.6	(−5.6; −3.6)	0.004	−1.3	0.064
	Above median (>6.0 mmol/L)	29	−0.41	(−0.45; −0.37)				−6.4	(−7.0; −5.8)			
Dose of PS	≥0.3 g/d and ≤1.5 g/d	13	−0.28	(−0.36; −0.20)	0.039	−0.07	0.034	−4.6	(−5.8; −3.3)	0.010	−1.2	0.011
	>1.5 g/d and <2.0 g/d	25	−0.35	(−0.41; −0.30)				−5.7	(−6.5; −4.9)			
	≥2.0 g/d and ≤3.2 g/d	17	−0.40	(−0.46; −0.35)				−6.8	(−7.6; −6.0)			

[a] P-value between subgroups <0.05 indicates a significant difference in pooled effect size between subgroups.
[b] P-value meta-regression <0.05 indicates a significant correlation between the variable under investigation and the effect sizes.

impact of these potential covariates on the absolute and relative changes in plasma PS concentrations could be detected (P-value >0.05). Regarding duration, we additionally analyzed whether there was a statistically significant difference between halfway and end-of-intervention plasma PS changes in studies that reported plasma PS concentrations at several time points [27,32,44,46]; again, no significant impact of duration was detected (P > 0.05).

Covariate analyses furthermore revealed that absolute and relative reductions in LDL-cholesterol and TC were larger with higher doses of PS and that absolute reductions in LDL-cholesterol and TC were larger with higher respective baseline concentrations. The impact of baseline cholesterol concentrations on the relative changes was weaker, especially for LDL-cholesterol (see Table 4).

At last, we analyzed whether the relative changes in LDL-cholesterol were related to the relative changes in plasma PS (Supplemental Appendix 6). In fact, no such correlation was found. Perhaps differences in metabolic fates between cholesterol and PS, e.g. circulating cholesterol is derived from synthesis and absorption whereas circulating PS can only be obtained through absorption, provide an explanation for this finding. Also, the considerable heterogeneity in plasma PS changes might have blurred the association with LDL-cholesterol.

4. Discussion

For the first time, the effect of PS-enriched food intake on plasma PS concentrations was systematically investigated by reviewing available data from published PS intervention studies. We observed significant increases in plasma sitosterol and campesterol concentrations after intake of PS-enriched foods, as was expected. The average increases in plasma sitosterol and campesterol concentrations were 2.24 μmol/L (31%) and 5.00 μmol/L (37%), respectively, with an average intake of 1.6 g/d PS. In the highest PS dose category (2.0–3.2 g/d), the average absolute increases were 3.56 μmol/L (42%) and 7.64 μmol/L (48%), respectively. The baseline plasma sitosterol and campesterol concentrations that we observed in the current meta-analysis (6.9 and 13.1 μmol/L, respectively) were comparable to what has previously been reported by Chan et al. [14]. In this review including data of 45 studies, average baseline concentrations for sitosterol and campesterol in the general population were 7.9 and 14.2 μmol/L, respectively. We furthermore observed an average reduction in LDL-cholesterol of 0.33 mmol/L (8.5%) which is similar to the pooled LDL-cholesterol-lowering effect expected for 1.6 g/d of PS based on several recent meta-analyses [3–5]. So, despite the smaller number of studies
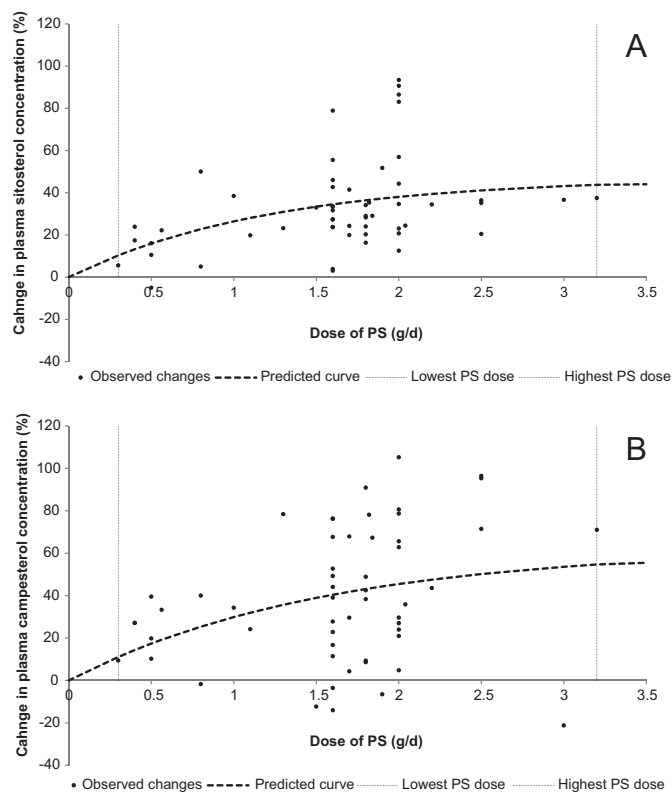
Fig. 3. Dose–response relationship between doses of PS and relative (%) changes in plasma sitosterol (Panel A) and campesterol (Panel B) concentrations. A first-order elimination curve was plotted through the observed changes.

(reporting plasma PS concentrations) included in the current meta-analysis compared to the more extensive cholesterol-lowering efficacy meta-analyses, our selection of studies seems representative for a broader range of studies. Also, with no less than 41 studies, a robust overview of the available literature has been developed.

In the current meta-analysis, the change in plasma PS concentrations was related to the dose of PS consumed per day, i.e. the higher the dose, the larger the increase in both sitosterol and campesterol concentrations. For PS-induced cholesterol-lowering, it is known that the decrease in plasma cholesterol concentrations would reach a plateau with increasing dose of PS due to saturable processes in cholesterol uptake and transport and subsequent feedback on cholesterol synthesis. Whether such tapering-off effect exists for plasma PS concentrations is yet unclear. In an attempt to investigate this, we established continuous dose–response curves for the relationship between PS doses and changes in plasma PS concentrations. These curves suggest that some tapering-off might exist although the maximal increase in plasma PS will likely be reached at doses higher than 3.2 g/d which was the highest dose tested in our meta-analysis. Studies investigating higher PS doses are scarce. Only two studies tested PS doses exceeding 3 g/d and reported serum PS concentrations. The study by Davidson et al. [69] tested PS intakes of 3, 6 and 9 g/d from enriched foods, but only reported medians and ranges of plasma PS concentrations. Based on their analysis, the increase in serum PS did not significantly differ between the three PS doses, except for the TC-standardized increase in campesterol. Noteworthy, even with the highest dose of PS (9 g/d), overall absolute PS concentrations remained below 2 mg/dL (~50 μmol/L). Another study by Tuomilehto et al. [70] investigated increasing intakes of PS (1.25, 2.5 and 5 g/d) together with a mix of minerals during three consecutive 5-week periods.

Serum sitosterol concentrations increased in a dose-dependent manner whereas no dose-dependent increase was observed in serum campesterol concentrations. From these data, together with the findings of the current meta-analysis which included studies investigating PS doses in the range of 0.3–3.2 g/d, no firm conclusions can be drawn on the dose–response behavior for plasma PS concentrations at higher PS doses (>3 g/d). The composition of the PS mixture, and related to this the PS source, also influenced the magnitude of the increase in plasma sitosterol and campesterol concentrations. For instance, studies that used PS derived from tall oil which contains less campesterol (~5–10%) and more sitosterol (75–80%) compared to e.g. soybean oil (20–30% campesterol and 45–50% sitosterol), showed smaller increases in plasma campesterol concentrations whereas increases in sitosterol concentrations were larger.

The concentrations of PS at baseline also seemed to explain part of the heterogeneity observed between different study results; in studies with higher average baseline PS concentrations, the absolute increase in plasma sitosterol and campesterol concentrations was larger compared to studies with lower average baseline concentrations. It could be that subjects with higher cholesterol/PS absorption efficiency (as indicated by higher baseline PS concentrations) are likely to absorb more PS when on PS intervention. Alternatively, the use of different analytical techniques to measure plasma PS concentrations could potentially have caused differences (systematic errors) in baseline concentrations and thus in changes upon intervention. This latter hypothesis is supported by the observation that baseline concentrations had no impact on relative changes in plasma PS concentrations which are less affected by systematic errors. Interestingly, Hendriks et al. [35] found that in subjects with the highest baseline PS concentrations, the average relative increase after one year consumption of PS-enriched margarine was even smaller as compared to subjects with lower baseline PS concentrations. This might suggest that some kind of feedback mechanism arises (e.g. upregulation of ABCG5/8) when PS are consumed for a longer period of time. Indeed, based on studies that reported plasma PS concentrations at different time points [27,32,44,46], the increase in plasma sitosterol and campesterol seemed to stabilize over time, which we confirmed in post hoc analyses. For example, in the study by de Jong et al. [27], plasma sitosterol and campesterol concentrations were similar after 45 weeks and 85 weeks of PS intervention.

The plasma PS concentrations that we observed in our meta-analysis are much lower than those reported in patients with homozygous sitosterolemia. Patients with this disease display plasma PS concentrations in the range of ~500–1200 μmol/L (~20–50 mg/dL) [22]. This is 20–45 times higher than the average total plasma PS concentration after intake of PS-enriched foods observed in the current meta-analysis. In this respect, the PS-induced increase in plasma PS can be considered modest and is not reaching the levels seen in homozygous sitosterolemics. Subjects with heterozygous sitosterolemia do not have such elevated plasma PS concentrations although their plasma PS concentrations are somewhat elevated (35–37%) compared to healthy controls [71]. These elevated concentrations are of the same order of magnitude as the increases in plasma sitosterol and campesterol concentrations after PS-enriched food intake seen in our study. Several studies [60,63,72,73] investigated what would happen if subjects with heterozygous sitosterolemia would regularly consume PS-enriched foods and found that these subjects showed similar plasma PS responses as compared to control subjects.

Recent evidence suggests that moderate, lifelong elevations in plasma PS concentrations in heterozygous sitosterolemics being carriers of the *ABCG8-G574R* variant are not associated with increased intima-media thickness (IMT). These subjects even

showed lower IMT compared to controls [71]. IMT is a commonly used predictor for CVD, although evidence does so far not convincingly support that progression of IMT is associated with CVD risk [74]. In contrast to the findings by Horenstein et al. [71], in a genome wide association study, gene variants in *ABCG8* were found to be significantly associated with increased serum PS concentrations and increased CVD risk [75]. However, as stated by Plat et al. [76], it cannot be ruled out that this association may be an epiphenomenon because plasma PS concentrations also reflect cholesterol absorption and, therefore, the association with CVD risk may be explained by increased absorption of cholesterol. Genser et al. [23] recently published a meta-analysis of observational studies that aimed to investigate the association between serum sitosterol and campesterol concentrations and CVD risk. The individual studies included in this meta-analysis showed conflicting evidence. However, based on seventeen studies reporting either plasma PS concentrations in CVD cases vs. controls or relative risks for CVD, it was concluded that, overall, no association between circulating PS (sitosterol and campesterol) and risk of CVD exists. In our meta-analysis, the observed control-adjusted average changes in plasma sitosterol and campesterol concentrations (2 and 5 µmol/L, respectively) were at least smaller than the difference between the upper and the lower tertiles of the sitosterol and campesterol distributions (6 and 10 µmol/L, respectively) reported in the Genser meta-analysis. Evidence from endpoint studies demonstrating a reduced risk of CVD has so far not been generated with intake of PS.

This meta-analysis has some limitations that need to be addressed. A considerable amount of heterogeneity was observed among the studies, more for circulating sitosterol and campesterol than for LDL-cholesterol and TC concentrations. Some of this heterogeneity could be explained by differences in PS dose, baseline PS concentration and in PS composition. However, many other factors could have induced variability between studies such as differences in study designs, test products and study populations. In particular, between-study differences in plasma PS concentrations may have been induced by differences in analytical methods used to measure plasma PS (i.e. differences in PS separation and detections methods). For better comparison between studies, there is a clear need for standardization of methods to measure plasma PS concentrations. Furthermore, the quality of the meta-analysis depends on the quality of the studies that have been included. As such, we had pre-defined rigorous selection criteria in order to exclusively retrieve studies that were suitable to answer our study objectives. We have not considered the quality of each individual study due to the rather subjective nature of such quality scoring. Finally, there is considerable indication that publication bias was present; the funnel plots suggested that studies reporting relatively small increases in plasma PS concentrations with low precision were lacking. In PS studies, determining changes in plasma PS concentrations is usually not the primary aim, and thus, it may well be that in some studies, blood samples were drawn to measure circulating PS but were eventually not analyzed or reported due to unknown reasons. In any case, given the observation that studies reporting relatively small increases in plasma PS seemed lacking, our findings are likely not underestimated. Despite these limitations, the current meta-analysis provides a good overview of all evidence available on this topic.

In summary, our meta-analysis including data from 41 randomized controlled studies showed that intake of PS-enriched foods (average PS dose was ∼1.6 g/d) increases circulating sitosterol and campesterol concentrations (2.24 and 5.00 µmol/L, respectively) whilst reducing TC and LDL-cholesterol concentrations (0.36 and 0.33 mmol/L, respectively). Overall, total PS remained below 1% of total sterols circulating in the blood which is far below levels seen in homozygous sitosterolemics. Since a considerable amount of heterogeneity was observed in plasma PS concentrations amongst the included studies, attempts to harmonize methods for measuring plasma PS concentrations should be undertaken.

Conflict of interest

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2013.08.012.

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