ORIGINAL CONTRIBUTION

The effect of plant sterols on serum triglyceride concentrations is dependent on baseline concentrations: a pooled analysis of 12 randomised controlled trials

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Abstract

Purpose Plant sterols (PS) are well known for their low-density lipoprotein cholesterol-lowering effect. Until recently, they were believed to have little or no impact on blood triglycerides (TG). However, studies taken individually were possibly lacking statistical power to detect modest TG decreases. This study was performed to quantify the TG-lowering effect of PS by pooling individual subject data from 12 randomised controlled trials that investigated the effects of PS on blood lipids.

Methods The main outcome variable was the control-adjusted PS effect on relative (%) and absolute (mmol/L) changes in TG. The relative and absolute changes in high-density lipoprotein cholesterol (HDL-C) were also assessed. Differences in changes of serum lipid concentrations between PS and control treatments were estimated by an ANCOVA using a random effect model which included PS intake (active or control), study and predefined subject characteristics.

Results The twelve randomised controlled trials included in total 935 hypercholesterolaemic subjects not preselected based on their baseline TG concentrations. In most studies, the PS dose ranged between 1.6 and 2.5 g/day. PS intake significantly lowered serum TG by 6.0% (95% CI: −10.7, −1.2) or 0.12 mmol/L (95% CI: −0.20, −0.04). No significant interaction was observed between PS intake and baseline TG concentrations on relative changes, but, on absolute changes, interaction was significant with larger TG decreases observed with higher TG concentrations at baseline. No effects were observed on HDL-C concentrations.

Conclusions These results show that PS exert a modest TG-lowering effect which is dependent on baseline concentrations.

Keywords Plant sterols · Triglycerides · Cholesterol · Pooled analysis · Diet and lifestyle

Introduction

Plant sterols (PS) and stanols, their saturated counterparts, are well known for their total and low-density lipoprotein cholesterol (LDL-C)-lowering effect. To date, several meta-analyses have summarised and quantified the LDL-C-lowering effect of PS/stanol-enriched foods and their dose–response relationship [1–4]. Possibly due to the fact that the large number of human intervention studies with PS/stanols were designed and powered to detect a significant effect on LDL-C, in most studies taken individually, the effect of PS/stanols on serum triglycerides (TG) was not estimated or not detected. However, significant reductions in TG concentrations after PS intervention have incidentally been observed [5–8]. Furthermore, a recent meta-analysis of individual subject data from five studies, which aimed at studying the relationship between subjects' baseline characteristics and the effects of plant stanol-enriched spreads on serum lipid concentrations, indicated that plant stanols not only lower serum concentrations of LDL-C, but also TG concentrations [9]. More recently, large TG reductions were observed in metabolic syndrome patients consuming PS/stanol-enriched foods [10, 11].

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Elevated TG concentrations are increasingly being recognised as a possible independent risk factor for coronary heart disease (CHD), and TG-lowering therapy next to lowering LDL-C may be considered relevant especially in high-risk populations such as, e.g., subjects with dyslipidaemia as characterised in the metabolic syndrome [12–14].

In the recent meta-analysis that indicated a TG-lowering effect of plant stanols [9], significant interaction was observed between baseline TG concentrations and plant stanol intake, resulting in larger TG reductions (expressed in mmol/L) with higher baseline TG concentrations. Even when expressed in terms of relative (expressed in %) changes from baseline, TG reductions were more pronounced when baseline TG concentrations were higher. For investigating the TG-lowering effect of PS, having individual subject data would thus allow making better adjustments for baseline TG concentrations resulting in more precise estimations. As such, the aim of the present study was to quantitatively evaluate the TG-lowering effect of PS by pooling individual subject data from randomised controlled trials that were made available by investigators from independent research groups.

In order to specifically take into account the baseline TG concentrations in the estimation of the TG-lowering effect, the main outcome was expressed as the relative change in TG from baseline values. In addition, and for better understanding the impact of baseline concentrations on the observed reductions in TG, the absolute changes were calculated. As HDL-C metabolism is closely related to that of TG via the action of the cholesterol-ester transfer protein (CETP) [15], the effect of PS-enriched food consumption on HDL-C concentrations was also evaluated.

Methods

Selection of the studies

Data sets of 14 Unilever-sponsored PS intervention studies published in 12 publications were made available by different independent research groups [5, 16–26] that published their findings in peer-reviewed journals. Studies were eligible for the current pooled analysis if they were randomised placebo-controlled trials with human adults not preselected based on their baseline TG concentrations, had used the 'usual' plant sterols (4-desmethylsterols), had disposal of TG data at baseline and at end of intervention as well as relevant co-variable data, and had no co-intervention from which the effect of PS could not be isolated.

Ferulated PS as found, e.g., in rice bran oil were excluded because these are not commonly used for food/supplement enrichment. In addition, there is no consensus on their cholesterol-lowering effect [16, 27]; thus, their potential impact on serum TG and/or HDL-C may also be different from that of other PS. Because the cholesterol-lowering effect of plant sterols is additive to that of statins [17, 28] and dietary fat modifications (diets low in total fat, saturated fat, and cholesterol content or high in vegetable oil) [29–31], we assumed that a similar additive effect could be expected in case of an impact on serum TG and HDL-C. Therefore, studies that prescribed statins or dietary fat modifications in both the control and the treatment group/phase within each study were included in the present analysis.

Eligibility for inclusion in the pooled analysis was judged by evaluating the full publication, the study protocol and the data set. Out of the 14 studies, one study was excluded because it did not measure TG concentrations [18] and another because initial lipid values were not readily available [19]. One study [23] consisted of two parallel arms with a randomised controlled cross-over design within each arm; these parallel arms were considered as two separate cross-over studies. In another study [24], 2 separate cross-over trials were described. Thus, individual subject data from a total of 12 studies from 10 publications that met the selection criteria were available for inclusion in the current pooled analysis [5, 16, 17, 20–26].

Data extraction and quality assessment

For each subject, the following data were extracted from the different data sets: study identification, gender, BMI, age, treatment (active or control), and TG and HDL-C data at baseline and at end of intervention. When the lipids were measured at various time points during the intervention, the values corresponding to or closest to the 4-week time point were taken for the analysis. If measurements were done on two different days at the end of the intervention, the mean value of those two measurements was taken.

Study quality was assessed as previously reported [3] using a custom-designed tool adapted from the Delphi Consensus [32] and the method by Chalmers et al. [33]. However, due to a lack of consensus on which scoring system is the best and hence scoring is intrinsically subjective [34], quality scores were not used to exclude lower quality trials or to weigh the data accordingly.

Statistical analysis

The primary outcome variables were the control-adjusted relative (%) and absolute (mmol/L) changes from baseline in TG due to the PS treatment. The secondary outcome

variables were defined as the control-adjusted relative and absolute changes from baseline in HDL-C. The relative changes in serum TG and HDL-C were calculated as follows for each subject:

$$\text{Relative change} = 100 \times \frac{\text{Lipid concentration}_{\text{end of intervention}} - \text{Lipid concentration}_{\text{start of intervention}}}{\text{Lipid concentration}_{\text{start of intervention}}}.$$

Baseline lipid concentrations were defined as the lipid concentrations at the start of the intervention phase (end of run-in when a run-in phase was present). For cross-over trials in which start-of-intervention measurements were not available ($n = 1$), the lipid concentrations at screening were used as baseline concentrations.

In order to standardise the variability structure of all data in the overall pooled analysis, we only used the data from the first study phase of cross-over studies, so that all studies were treated as parallel studies.

For the absolute changes, analysis was done on end-of-intervention serum lipid concentrations while adjusting for baseline concentrations. Differences in mean relative changes and absolute serum TG and HDL-C concentrations between the PS group and the control group were determined by an ANCOVA using a model which initially included PS intake (active or control), study and the pre-defined subject characteristics age, gender, BMI and baseline lipid concentrations and their interactions with PS intake. Because age and gender did not significantly ($P > 0.1$) contribute to the model, the subject characteristics kept in the final model were the respective baseline lipid concentrations and BMI (and the interaction between baseline TG concentrations and PS intake in the case of absolute changes). The statistical analysis was performed for the quasi intention-to-treat population [35], i.e., using all subjects for whom end-of-intervention TG or HDL-C values were available, and according to a random effect model.

Sensitivity analysis was performed to determine whether the presence of one study with patients on statins [17] influenced the outcome. The effect of PS on TG and HDL-C (expressed as relative change) was thus also determined when using only the eleven studies with healthy subjects. In order to verify that the use of only the first phase of cross-over trials in the overall analysis did not affect the outcome, a separate analysis was performed by using all phases of the cross-over trials.

Heterogeneity between studies was assessed by calculating the Q statistic as described by DerSimonian and Laird [36].

All analyses were performed with the statistical software program The SAS System (SAS Version 9.2, SAS Institute, Inc., Cary, NC, USA). ProcMixed was used to perform the analyses.

Results

Overview of included studies and subjects

In total, 12 studies from 10 publications were available for the current pooled analysis [5, 16, 17, 20–26]. The study by Noakes et al. [24] included PS and plant stanol treatments; only the data from the PS arm were used. When parallel design studies included different PS treatments (e.g. PS from different sources) provided in the same food format, these strata were combined [5, 22]. In all studies, blood lipid concentrations were measured after an overnight fast. TG concentrations were included in the eligibility criteria of 9 out of 12 studies and were defined as less than 3.4–4.5 mmol/L in most ($n = 8$) studies. Table 1 shows the characteristics of the studies included. The majority of studies was judged as of good quality (data not shown).

PS were esterified to vegetable oil fatty acids in all studies except one [16] which used free PS. The food format was margarine or spread in the majority of studies ($n = 9$). In one study, a combination of spread and milk ($n = 1$) was used [25], and in two studies, the vehicle for PS was a salad dressing [23]. The PS dose varied between 0.8 and 4 g/day, with the majority of studies ($n = 9$) testing doses ranging between 1.6 and 2.5 g/day. Doses of 0.8, 1.3 and 4 g/day were used in the other studies [16, 25, 26]. In most cases, PS-enriched foods were consumed for a period of 3 weeks; in three studies, the treatment duration was longer than 4 weeks, namely 5, 8 or 52 weeks [17, 20, 22]. In these cases, data obtained at 3 or 4 weeks were used in order to standardise the data from all studies to a similar point in time after the start of the intervention. Frequency of test product intake was not reported in three studies [16, 25, 26], whereas PS were consumed 2–3 times/day with meals in the other studies. Subjects were allowed to keep their usual, self-selected diet during the intervention in half of the studies [5, 16, 17, 20, 21, 25]. In the other studies, the subjects were either provided a typical North-American diet [23] or were advised to follow the NCEP Step 1 diet [22, 26] or to consume a diet rich in carotenoid-rich fruits and vegetables [24].

A total of 935 participants were included in the current pooled analysis. In 11 of the 12 studies, the subjects were overall healthy and were not taking any lipid-lowering medication. The only exception was the study by Neil et al. [17] in which subjects received statins and half of them had familial hypercholesterolaemia. In all studies, subjects were Caucasian. The mean age of the study populations varied between 44 ± 12 and 58 ± 11 years. On average, the subjects were slightly overweight (mean BMI ranging between 24.0 ± 2.9 and 27.3 ± 3.7 kg/m^2). Mean baseline TG concentrations were on average normal to borderline high (ranging from 1.37 ± 0.52 to 1.93 ± 1.08 mmol/L)

Table 1 Overview of the studies included in the current study

Author and reference	Original design	Sample size Control n	Sample size Plant sterols n	Mean age (y)	Mean BMI (kg/m²)	Male (%)	Triglyceride eligibility criteria (mmol/L)	Mean baseline TG (mmol/L)	Mean baseline Total-C (mmol/L)	Mean baseline LDL-C (mmol/L)	Mean baseline HDL-C (mmol/L)	Food format	Dose (g/day)	Duration (wks)[b]
Sierksma et al. [16]	Cross-over[c]	26	24	44.3 ± 11.6	24.9 ± 2.3	52	None	1.37 ± 0.52	5.09 ± 1.07	3.15 ± 0.86	1.33 ± 0.44	Margarine	0.8[d]	3
Maki et al. [22]	Parallel	89	131	58.0 ± 10.7	27.3 ± 3.7	44.8	<4.0	1.59 ± 0.73	6.17 ± 0.76	4.13 ± 0.67	1.32 ± 0.36	Margarine	1.1 or 2.2	4
Neil et al. [17]	Parallel	29	29	50.2 ± 12.7	26.1 ± 4.2	43.1	≤3.5	1.48 ± 0.76	7.29 ± 1.09	5.11 ± 1.07	1.45 ± 0.32	Margarine	2.5	3.5
Judd et al. study 1 [23]	Cross-over	12	15	47.9 ± 12.5	27.0 ± 2.8	51.9	<3.4	1.46 ± 0.73	5.76 ± 0.75	3.68 ± 0.57	1.42 ± 0.44	Salad dressing (Ranch)	2.2	3
Judd et al. study 2 [23]	Cross-over	14	12	46.4 ± 9.9	27.0 ± 3.0	46.2	<3.4	1.49 ± 0.69	5.48 ± 0.64	3.57 ± 0.53	1.24 ± 0.32	Salad dressing (Italian)	2.2	3
Mussner et al. [21]	Cross-over	31	31	–	24.0 ± 2.9	–	<1.8	1.19 ± 0.37	6.16 ± 0.71	4.11 ± 0.62	1.51 ± 0.35	Margarine	1.8	3
Noakes et al. study 1 [24]	Cross-over	14	18	57.5 ± 8.3	25.8 ± 2.9	40.6	<4.5	1.55 ± 0.54	6.31 ± 0.67	4.44 ± 0.57	1.17 ± 0.33	Margarine	2.3	3
Noakes et al. study 2 [24]	Cross-over	16	19	57.3 ± 9.7	26.0 ± 2.4	57.2	<4.5	1.73 ± 0.93	6.06 ± 0.71	4.20 ± 0.58	1.12 ± 0.24	Margarine	2.0	3
Hendriks et al. [20]	Parallel	97	91	48.5 ± 7.7	24.8 ± 3.2	48.4	None	1.35 ± 0.71	6.01 ± 1.01	3.79 ± 0.97	1.65 ± 0.41	Margarine	1.6[d]	3
Colgan et al. [26]	Cross-over	17	30	46.0 ± 8.8	26.2 ± 3.2	55.3	None	1.44 ± 0.65	6.23 ± 0.66	4.01 ± 0.79	1.18 ± 0.35	Margarine	1.3[d]	3
Noakes et al. [25]	Cross-over	21	18	51.5 ± 11.2	26.0 ± 2.1	53.9	≤4.5	1.67 ± 0.71	6.83 ± 0.82	4.83 ± 0.79	1.26 ± 0.39	Spread and milk	4.0	3
Clifton et al. [5]	Parallel	39	112	54.0 ± 8.7	26.5 ± 3.4	56.6	<4.5	1.93 ± 1.08	6.48 ± 0.77	4.34 ± 0.80	1.45 ± 0.48	Margarine	1.6	3

[a] Data are means ± SD unless mentioned differently

[b] Data at the time point closest to 4 weeks were taken

[c] For cross-over studies, only the 1st phase was used in the current study and treated as a parallel design

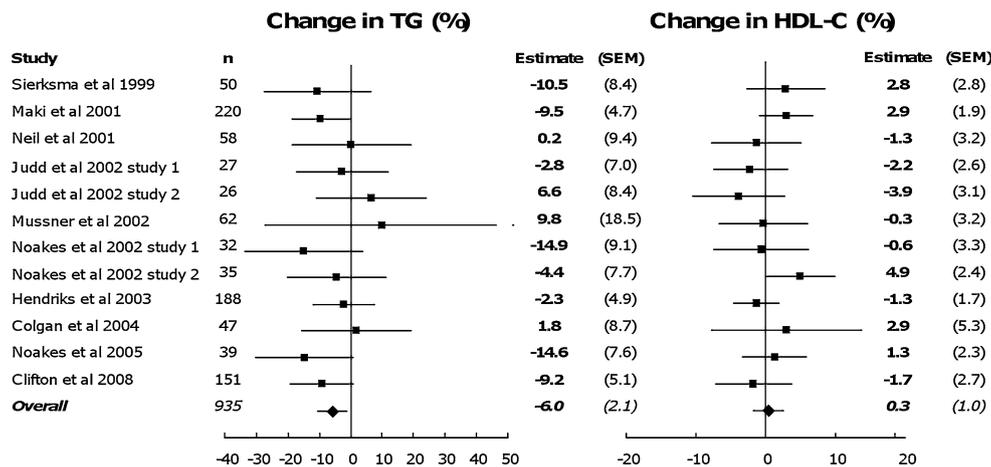[d] Compliance data were available to determine the dose truly consumed

Fig. 1 Forest plots. Forest plots showing the effect of plant sterols on TG and HDL-C estimated for each of the studies included in the overall analysis using individual subject data. The *squares* represent the averages for each of the individual studies. *Error bars* represent 95% confidence intervals (CIs). The *diamonds* represent the pooled results. The *solid vertical line* extending upward from zero is the null value. In both the overall and individual study analyses, only the first phase of cross-over trials was used. Both types of analyses were performed using individual subject data. The overall estimate was obtained by pooling together the individual subject data from all studies. The same statistical model was used for the individual studies and the overall analysis; the model included PS intake, study, age, gender, BMI and the respective baseline concentrations and their interactions with PS intake

according to the NCEP classification [14], whereas LDL-C concentrations were on average above optimal to very high (ranging from 3.15 ± 0.86 to 5.11 ± 1.07 mmol/L). The baseline characteristics of the subjects in each of the studies are presented in Table 1.

Heterogeneity analysis

For the relative changes in TG, there was no significant heterogeneity between the studies as assessed by the Q statistic ($Q = 0.22$, 11 degrees of freedom, $P > 0.95$). For HDL-C, no significant heterogeneity was observed either ($Q = 2.18$, 11 degrees of freedom, $P > 0.95$).

TG outcomes

When combining the individual subject data from all studies, PS significantly lowered serum TG by 6.0% (95% CI: $-10.7, -1.2$, $P = 0.02$) (Fig. 1). No significant interaction was observed between TG effects of PS intake and baseline TG concentrations ($P = 0.38$).

When the study with statin users [17] was removed from the analysis, the pooled estimate was a 6.3% reduction in TG (95% CI: $-11.3, -1.3$, $P = 0.02$). An analysis of only cross-over studies including all treatment phases showed a similar effect, namely a 5.6% reduction in TG (95% CI: $-9.3, -2.0$). The ANCOVA performed for each study separately showed non-significant TG reductions in 8 out of 12 studies (Fig. 1).

When the effects were expressed in absolute values, PS intake modestly but significantly lowered TG by 0.12 mmol/L
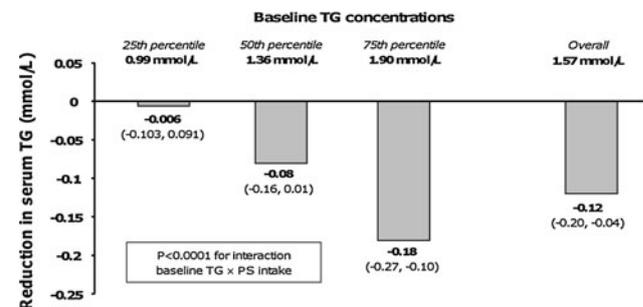

Fig. 2 Impact of baseline TG concentrations. Impact of baseline TG concentrations on the absolute (expressed in mmol/L) TG reductions achieved with PS consumption in twelve randomised controlled trials. In the majority of studies ($n = 9$), doses of 1.6–2.5 g/day were tested (range: 0.8–4.0 g/day)

(95% CI: $-0.20, -0.04$, $P = 0.01$). In contrast with the results obtained when the effects were expressed relatively, a significant ($P < 0.01$) interaction between PS intake and baseline TG concentrations was observed on absolute end-of-intervention concentrations. In line with this finding, larger reductions versus control were observed in subjects with higher baseline TG concentrations (Fig. 2).

HDL-C outcomes

No significant effect of PS was observed on HDL-C; the relative change from baseline was +0.3% (95% CI: $-1.8, +2.5$, $P = 0.73$) (Fig. 1). There was no interaction between PS intake and baseline HDL-C concentrations ($P = 0.75$). The removal of the study with statin users [17] did also not have an impact (HDL-C change = +0.5, 95% CI: $-1.8, +2.8$, $P = 0.66$).

When the analysis was performed on the absolute HDL-C concentrations, also no significant effect of PS intake was observed (+0.01 mmol/L; 95% CI: −0.02, +0.04, $P = 0.54$) and there was no PS intake × baseline HDL-C interaction ($P = 0.44$).

Discussion

The present pooled analysis including individual subject data from 12 randomised controlled trials shows that PS intakes of around 2 g/day exert a modest TG-lowering effect of about 6% or 0.12 mmol/L in hypercholesterolaemic subjects not preselected based on their baseline TG concentrations. Given the high inter-individual variation in TG concentrations, and the fact that the individual PS studies were primarily powered to assess the effect of PS on LDL-C concentrations, it is likely that the absence of statistically significant TG-lowering effects in these studies was due to insufficient statistical power. For example, a recent study by Mensink et al. [37] studied the serum lipid effects of doses of plant stanols up to 9 g/day but failed to show a significant TG reduction (e.g. ∼8% for 9 g/day; $P = 0.187$) with only a limited number of subjects in each of the treatment groups (∼22 to 25 subjects).

The 6% TG-lowering effect observed here is consistent with the outcome of a previous meta-analysis of individual subject data from five studies [9] which showed a 4% reduction in TG after 2 g/day plant stanol intake in subjects with baseline concentrations of ∼2 mmol/L. These data thus show that both PS and stanols exert a comparable TG-lowering effect. Other recently published studies using similar doses of PS (∼2 g/day) also support the findings of our pooled analysis; TG concentrations were significantly lowered by 9–19% after 4–6 weeks of intervention with PS-enriched (soy)milk or spread in subjects with baseline TG concentrations >1.5 mmol/L [5–8]. For plant stanols as well, significant decreases in TG concentrations were shown in subjects with overt hypertriglyceridaemia [38].

The TG-lowering effect observed in our pooled analysis seems robust. Heterogeneity analysis did not reveal significant variability between studies. In addition, the sensitivity analysis showed that removing the study with statin users did not affect the outcome. Also, the use of only the first phase of cross-over trials in the overall analysis did not change the results. At last, the majority of studies included in the pooled analysis were of good quality, and most individual studies showed a tendency towards the same direction in the form of non-significant TG reductions.

Our results indicate that the absolute (mmol/L) reductions in TG achieved with PS intake are dependent of baseline TG concentrations. A significant interaction on relative (%) TG changes was not present. However, it cannot be fully excluded that the current analysis may have been under-powered to detect such an effect. Nevertheless, the present results suggest that the impact of baseline TG is more pronounced on absolute changes in TG concentrations than on relative changes from baseline. By expressing TG changes as % change from baseline, at least part of the variability in PS effects due to inter-individual variations in baseline TG is taken into account. Therefore, it appears preferable to express the TG changes in relative terms when referring to the mean effect in a population.

Our data fit well with the findings of two studies reporting large control-adjusted TG reductions of 19–28% (corresponding to 0.23 to ∼0.4 mmol/L) following the consumption of 2–4 g/day PS/stanols in metabolic syndrome subjects with baseline TG concentrations of 2.2–2.4 mmol/L [10, 11]. We estimated, for our study population, a reduction of 0.18 mmol/L in subjects with baseline TG concentrations at the 75th percentile (1.9 mmol/L). If our pooled analysis had comprised a larger proportion of subjects with higher baseline TG concentrations and/or subjects with the metabolic syndrome, it is likely that even larger TG reductions would have been observed. Taken together, these data suggest that PS/stanols would be particularly useful for a dual benefit on both LDL-C and TG in subjects with both lipid abnormalities.

Based on the significant reductions in large and medium size VLDL particles observed in subjects with the metabolic syndrome, Plat et al. [39] suggested that a reduced hepatic VLDL1 secretion could be a mechanism involved in the TG-lowering effect of plant stanols. The unaltered CETP mass observed in their subjects coupled with unchanged HDL-C concentrations [39] is consistent with the absence of effect of PS on HDL-C observed in the present study. Overall, these data suggest that the reduced TG concentrations attributable to either PS or stanol consumption may not be ascribed to a remodelling of TG-rich lipoproteins via CETP activity.

The findings of the current pooled analysis are limited by the fact that the randomised controlled trials included in the analysis present only a selection of studies available in the literature. Also because the included studies were all industry-sponsored, selection bias might possibly be present. However, all studies were planned and executed by independent research groups and published in peer-reviewed journals. Because we re-analysed individual subject data of a large number of subjects (935 in total), we believe that there was sufficient power to substantiate the conclusions drawn and that adding more subject data from other studies would not have changed the outcomes. In addition, because most studies used PS doses within a narrow range (between 1.6 and 2.5 g/day), this does not allow drawing any conclusion on a possible dose–response relationship for the TG lowering effect of PS.

In the absence of intervention studies that directly quantified the CHD risk reduction resulting from lowering TG only, it is difficult to determine whether the additional effect that a modest 6% TG reduction may have on CHD risk is clinically relevant next to the average 10% LDL-C reduction achievable with an intake of 2 g/day of PS. Nevertheless, although not as strong as LDL-C, elevated TG is increasingly being recognised as a possible risk factor for CHD [12–14]. Additional research into the relevance of TG lowering for CHD risk reduction, and into interventions (e.g. diet and lifestyle interventions) that beneficially impact TG, is therefore warranted.

In conclusion, foods enriched with PS modestly lower TG concentrations, especially in those with high TG concentrations at baseline. This effect may add to the overall benefit of using PS-enriched foods as part of therapeutic lifestyle and diet changes for improving blood lipid profiles.

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