Fat type in phytosterol products influence their cholesterol-lowering potential: A systematic review and meta-analysis of RCTs.

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Abbreviations: ACAT, acyl coenzyme A:cholesterol acetyltransferase; ALA, alpha-linolenic acid; apo, apolipoprotein; CHD, coronary heart disease; CVD, cardiovascular disease; CYP7, cholesterol 7α-hydroxylase; D, dairy fat; HDL-C, high-density lipoprotein cholesterol; HNF-4, hepatic nuclear factor-4; LA, linoleic acid; LDL-C, low-density lipoprotein cholesterol; LDL-r, low-density lipoprotein receptor; LXR, liver X receptors; MUFA, monounsaturated fatty acid; NPCL1L1, Niemann-Pick C1-Like1; n-3, omega-3; n-6, omega-6; PS, phytosterols/phytostanols; PPAR, peroxisome proliferator activated receptors; PUFA, polyunsaturated fatty acid; RC, rapeseed/canola fat; RCT, randomised control trial; SFA, saturated fatty acid; SREBP, sterol regulatory element binding proteins; SS, sunflower/soybean; TC, total cholesterol; VLDL-C, very low-density lipoprotein cholesterol.

ABSTRACT

The most common form of phytosterol (PS) fortified foods are fat spreads and dairy products. The predominant fats used are soybean/sunflower (SS) or rapeseed/canola (RC) oils and animal fat (D) in dairy products. This review aimed to investigate whether the carrier fat is a determinant of the hypocholesterolaemic effects of PS fortified foods. Databases were searched using relevant keywords and published RCTs from 1990 investigating the effects of dietary PS intervention (≥1.5 g per day) on total cholesterol and LDL-C were included. After methodological quality assessment and data extraction, a total of 32 RCTs (RC, n=15; SS, n=9; D, n=8) were included. As expected, all fat groups significantly reduced TC and LDL-C ($p<0.01$). When compared across different carrier fats, RC as the main carrier fat, reduced LDL-C significantly more than the SS spreads ($p = 0.01$). Therefore, a combination of monounsaturated fatty acid rich spread with adequate amounts of omega-3 fatty acids (as evident in RC spreads) may be the superior carrier fat for the delivery of PS for optimal blood cholesterol-lowering. The findings of this research provide useful evidence for optimising the hypocholesterolaemic effects of PS and support further investigation into the possible mechanisms behind these findings.

Keywords: phytosterols, rapeseed, canola, sunflower, dairy, cholesterol

Contents

1. INTRODUCTION 4
1.1. Phytosterols 4
1.2. Dietary fats 6
1.3. Mechanisms by which dietary fats modulate blood lipid levels 7
1.4. Potential synergistic and complementary effect of phytosterols and dietary fats 10
2. MATERIALS AND METHODS 11
2.1. Search strategy 11
2.2. Eligibility Criteria 11
2.3. Selection process and quality assessment 12
2.4. Data extraction 13
2.5. Statistical analysis 13
3. RESULTS 15
3.1. Overview of publications 15
3.2. Description of publications 15
3.3. Effect of fat type on the phytosterol-lowering of blood cholesterol levels 16
4. DISCUSSION 18
5. CONCLUSION 23
6. ACKNOWLEDGEMENTS 24
7. REFERENCES 25
8. TABLES 32
9. FIGURES 41
10. FIGURE LEGENDS 44

1. INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality, claiming 31% of all deaths worldwide in 2012 [1]. The global economic impact of CVD is estimated to be US $906 billion dollars in 2015 and is expected to rise by 22% by the year 2030 [2]. Elevated total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) concentrations in the blood are major diet-related risk factors for the development of CVD [3].

1.1. Phytosterols

It is well established that phytosterols and their saturated form, phytostanols, are key cholesterol-lowering agents. PS (comprising phytosterols and phytostanols) are non-nutritive compounds similar in structure to cholesterol, however, are produced by plants and are essential components of cell membranes. PS are solely derived from the diet and are naturally found in plant based foods such as vegetable oils, fruits, nuts, seeds, legumes and fat spreads [4]. Western populations are estimated to consume 150-450 mg/day of phytosterols in the diet, with Japanese and vegetarian people consuming up to 50% more. Moreover, only 20-50 mg/day of phytostanols are consumed from foods on a regular basis [5]. In addition to natural sources, staple foods have been fortified with PS, the most common being fat spreads and low-fat dairy products [4]. Other products include breakfast cereals, cereal bars, orange juice, chocolate, muffins, croissants, breads, vegetable oils, salad dressings, mayonnaise and tortilla chips [6]. Intestinal absorption of PS compared to dietary cholesterol is markedly less (0.5-2% vs 55-60%) [7, 8], with the majority being rapidly excreted by the liver [9].

PS differ from cholesterol by containing an extra ethyl (β-sitosterol) or methyl (campesterol) group at C-24 or an extra double bond (stigmasterol) at C-22 [9]. Dietary and biliary PS and cholesterol must be solubilised in micelles prior to digestion and intestinal absorption. The micelle interacts with the brush border membrane of the intestinal lumen for sterol uptake, which is facilitated by transport protein Niemann-Pick C1-Like1 (NPC1L1)

[7]. ATP-binding cassette proteins (ABCG5 and ABCG8) shuttle any unesterified cholesterol and PS back to the intestinal lumen [7].

Dietary PS can reduce cholesterol absorption by 30 to 50% [10]. Several mechanisms have been proposed, however, the most widely accepted mechanism involves PS displacement of cholesterol in the micelle due to the greater affinity of PS [11]. This causes cholesterol to precipitate out into the lumen, hence limiting intestinal solubility of cholesterol and hydrolysis of cholesterol esters in the intestines [4, 12]. PS may also enhance the efflux of cholesterol via inducing the expression of ABCA1 transporter which cannot differentiate between cholesterol and PS [13]; in addition, PS may suppress acyl coenzyme A:cholesterol acyltransferase (ACAT) activity leading to a subsequent reduction in cholesterol uptake/transport in the intestine [9].

PS were first reported to play a regulatory role in serum cholesterol concentrations in 1951 [14] following by a number of clinical trials to establish that 2.0-2.5 g/day of PS elicit a 10-15% reduction in plasma LDL-C concentrations within 2-3 weeks [9, 15]. Given that sterols require fat for solubilisation, a practical mode of delivery for PS esters has been their dispersion into fat spreads. This elicits an optimum transport vehicle which increases lipid solubility and promotes PS incorporation into micelles [9]. A meta-analysis of 124 randomised control trials (RCT) showed that a PS intake of 0.6-3.3 g per day dose-dependently lowers LDL-C concentrations by 6-12% [16]. This reduction is considered clinically significant as it has been demonstrated that a 1% reduction in LDL-C leads to a 1% decrease in coronary heart disease (CHD) risk [17].

There are some factors known to influence PS efficacy such as dosage, baseline LDL-C concentrations, lipid-lowering medication and combination therapy with other nutraceuticals such as omega-3 polyunsaturated fatty acids (n-3PUFA). As mentioned above, PS dose-dependently lower LDL-C concentrations with greater reductions seen in those with higher

baseline LDL-C concentrations [6]. A meta-analysis on continuous dose-response relationships of the LDL-C-lowering effect of PS showed that the total fat content of the meal, PS type (sterols vs stanols) and dairy vs non-dairy food vehicles, did not significantly affect the LDL-C lowering efficacy of PS [6]. Combining PS supplementation (~2.5 g/day up to 5.0 g/day for 4 to 8 weeks) with statin therapy resulted in additive reductions in LDL-C by 10-15% [18-20] compared to a 5-7% reduction when the statin dose was doubled [21].

Long-term clinical trials are yet to report any serious adverse side effects of long-term PS consumption, however, a common observation is a modest reduction of plasma carotenoid levels by up to 10% [22]. This is offset by increasing fruit and vegetable consumption by one extra serve per day whilst consuming the cholesterol-lowering dosage of PS [22]. A rare condition associated with PS is called phytosterolaemia, involving dysfunction in genes coding for the ATP-binding cassette transporters ABCG5 and ABCG8, key regulators of PS absorption.

1.2. Dietary fats

It is well known that dietary fats modulate circulating lipid concentrations and are associated with CVD risk [23]. The classically held belief backed by over half a century of interventional and epidemiological studies acknowledges saturated fats as the major causative factor for the development of CHD and source of morbidity and mortality in the Western world [24]. Despite this public and scientific belief, conclusive evidence confirming the exact relationship between dietary saturated fatty acids (SFA) and blood cholesterol concentrations remains controversial [24]. Recent studies have suggested that the food source and type of SFA are more important determinants of their lipid modulating potential [24]. For instance, dairy fats containing short chain fatty acids (less than 6 carbon atoms) may not be as hypercholesterolaemic as tallow or lard providing longer chain fatty acids (greater than 14

carbon atoms) [25-28]. A number of studies have shown that trans fatty acids (TFA) increase plasma LDL-C and lipoprotein (a) and lower HDL-C [23, 29] with subsequent mortality from coronary artery disease [23]. On the other hand, cis isomers of monounsaturated fatty acids (MUFA) have been shown to reduce LDL-C and increase HDL-C concentrations [30-34] as well as modulate some markers of inflammation [30]. PUFA of the omega-6 (n-6) family are more effective in lowering circulating TC and LDL-C concentrations, but have no effect on TG or HDL-C [23]. Some researchers have raised concerns about the health effects of n-6PUFA due to their potential to promote pro-inflammatory mediators [9]. Conversely n-3PUFA, particularly of marine origin (eicosapentaenoic acid and docosahexaenoic acid) are potent lipid (TG)-lowering agents and possess mild LDL-C and HDL-C raising effects while shifting the LDL particle size to larger, buoyant and less atherogenic particles [9, 23].

The importance of exploring interactions between various classes of dietary fats rather than the effects of a single dietary fat in isolation for optimal blood lipid profile and subsequent reduced CVD risk cannot be undermined.

1.3. Mechanisms by which dietary fats modulate blood lipid levels

The current evidence exploring the mechanisms by which dietary fats modulate circulating blood lipids is largely limited to animal models. Fernandez et al [23] demonstrated that dietary fatty acids modulate circulating LDL-C by interfering with LDL receptor (LDL-r) activity, protein and mRNA abundance. Moreover, dietary fatty acids modulate plasma cholesterol indirectly via regulation of four families of transcription factors: peroxisome proliferator activated receptors (PPARs), liver X receptors (LXRs), hepatic nuclear factor-4 (HNF-4) and sterol regulatory element binding proteins (SREBPs) [23].

Dietary SFA have been shown to impose their LDL-C-raising effects by inhibiting LDL-r activity and promoting apolipoprotein (apo) B-containing lipoprotein production [35]. The

degree of LDL-C raising appears to be more pronounced with higher dietary cholesterol intake [35]. Longer chain SFA (e.g. stearic acid) have shown no effects on blood lipids, however, SFA of shorter chain length such as myristic and palmitic acid have been shown to induce a rise in LDL-C [23, 27, 36]. Negligible effects on LDL-C clearance and concentrations in non-human primates administered a high SFA diet are evident in the absence of dietary cholesterol and adequate intake of PUFA [37]. LDL-r activity is suppressed in humans and animals by SFA (particularly palmitic acid) upon an energy dense and dietary cholesterol-rich, diet [37]. The LDL-r plays an important role in the delivery of cholesterol to the cell, therefore, any reduction in LDL-r activity results in a subsequent accumulation of cholesterol in circulation. SFA are known to supress the rate-limiting enzyme known as ACAT, which is involved in regulating cholesterol esterification. *In vitro* and animal studies have suggested that dietary fatty acids and cholesterol modulate LDL-r activity in the liver via cholesteryl ester and unesterified cholesterol pools [38-40]. Given ACAT's regulatory role in cholesterol pools, and SFA suppression of its activity, a larger proportion of cholesterol may remain in these pools, therefore reducing LDL-r activity and subsequently contribute to elevated levels of LDL-C as observed in SFA-rich diets [23]. It is important to note that the heterogeneity of responses to dietary SFA intake may be explained by intrinsic differences in the regulation of lipid metabolism for e.g. obesity, insulin resistance, female gender, hypertriglyceridaemia and apoE polymorphism [36].

PUFAs have been shown to increase LDL-r activity, protein and mRNA abundance via possible modulation of hepatocyte membrane fluidity [23]. In humans and non-human primates, dietary MUFA reduced LDL-C without lowering HDL-C, and when compared to SFA and PUFA, dietary MUFA result in lowest LDL: HDL ratio [34, 41, 42]. Human studies have reported mixed results regarding the TC and LDL-C lowering effects of MUFA compared to PUFA. Some report comparable cholesterol-lowering effects, whilst others have

reported a greater effect induced with PUFA [43]. A MUFA-rich diet derived from plant sources may offer additional benefits to lipid profile by improving the quality of LDL-C. Human studies have shown that LDL-C in individuals consuming MUFA-rich diets were less susceptible to oxidation than those who consumed a PUFA diet, irrespective of the total fat content in the diet i.e. low-fat vs high-fat [44].

Dietary n-6PUFA play an independent regulatory role on LDL-r expression, with an upregulation of LDL-r seen in animals fed a diet containing 0.25% total energy as cholesterol. Compared to SFA (palmitic acid), n-6PUFA upregulated LDL-r protein relative to controls on a low-fat/cholesterol-free or cholesterol-only diet [45]. LXR regulates intracellular cholesterol levels by inducing the expression of cholesterol 7α-hydroxylase (CYP7); the rate-limiting enzyme required for the conversion of cholesterol to bile acids. PUFA have been shown to induce LXR expression in rats [46], thus resulting in an indirect increase in CYP7 activity causing elimination of cholesterol from the liver and a subsequent reduction in plasma cholesterol levels. Animal and *in vitro* studies have demonstrated a reduction in LDL-C apoB pool size by 50% and a two-fold increase in LDL-C fractional catabolic rate following administration of PUFA when compared to SFA, hence having an overall favourable effect on LDL-C clearance [23]. Dietary n-3PUFA confer additional regulatory benefits during cholesterol metabolism. Many human studies report decreased residence time of very low-density lipoprotein cholesterol (VLDL-C) in serum after dietary supplementation with n-3PUFA [23, 47].

The majority of the available evidence for the mechanistic properties of dietary fats and their modulation of blood lipids is based on animal or cellular models conducted up to 30 years ago. Recent human data are warranted to elucidate the exact mechanisms involved in dietary fat modulation of blood lipids in order to confirm the previous observations of animal and experimental models. Rigour in study design including appropriate placebo-controlled

groups, focus on whole-food consumption, adjustment for confounders and the assessment and analysis of diet is crucial for the determination of how dietary fats are regulating circulating blood lipid concentrations.

1.4. Potential synergistic and complementary effect of phytosterols and dietary fats

The mechanisms by which PS lowers blood cholesterol levels are different from those of dietary fats. Moreover, the efficacy of PS to reduce circulating levels of cholesterol may depend on the fat content of the meal and fat spreads are convenient means of delivering the two together. The predominant fats used in the PS enriched fat spreads are soybean/sunflower oil (SS) or rapeseed/canola oil (RC) and dairy fat (D) in dairy products. SS oil based spreads are predominately composed of linoleic acid (LA) and RC oil based spreads are predominately composed of oleic acid with considerably higher amounts of alpha-linolenic acid (ALA) than the SS oil based spreads [48]. Dairy based PS enriched food products are composed predominately of SFA, with relatively small amounts of PUFA or MUFA.

The purpose of this systematic review and meta-analysis was to determine whether the type of carrier fat used in PS enriched fat spreads and dairy products, is a determinant of the hypocholesterolaemic effects of PS enriched products. The systematic review aims to underpin the mechanisms by which PS and dietary fats influence blood cholesterol levels, with particular reference to the interactions between the two. The meta-analysis of the RCTs conducted using the various PS fortified food products, will provide an in-depth understanding of how circulating lipid levels may be optimised by using PS in combination with different dietary fats.

2. MATERIALS AND METHODS

2.1. Search strategy

A research librarian assisted with the planning of the systematic search for publications in May 2015. Four electronic databases (EMBASE, MEDLINE, CINAHL and Cochrane Library) were searched from end-May 2015 to mid-August 2015. The following Medical Subject Headings (terms), words and/or their combinations were searched: phytosterol, phytostanol, plant sterol(s), plant stanol(s), sitosterol, sitostanol, campesterol, campestanol, stigmasterol, stigmastanol; and randomised controlled trial, randomized controlled trial, intervention study, intervention studies, clinical trial(s), random*, group*, trial*. Keywords were searched in the title, abstract or topic as free text and combined using the Boolean operator 'AND'. To optimise publication retrieval, the internationally broad MeSH terms and spelling were used. Limits included publications from 1990 to present, English language, humans and adults. Additional publications were identified from the reference lists of included papers and systematic reviews retrieved by the initial search. Outcomes were divided into two groups: TC and LDL-C.

2.2. Eligibility Criteria

Table 1 shows the inclusion and exclusion criteria for the selection of publications. Publications that did not report cholesterol and/or LDL-C as an outcome measure were excluded from this review. Publications that reported blood cholesterol values in mg/dL were converted to mmol/L using the standardised conversion method (multiply mg/dL by 0.0259) [49]. When fat type of the intervention product was not stated in the paper, authors of publications were contacted. Publications could not be included in this review if the fat type was unable to be ascertained and when authors of the paper could not be contacted or could not provide or confirm the fat type.

2.3. Selection process and quality assessment

The title and abstract of publications were screened (JF) for the first selection process based on the eligibility criteria (Table 1). Studies that included a form of cholesterol-lowering therapy (i.e. statin) were only selected if they involved PS-alone treatment arms (i.e. PS) as well as a control or placebo group without cholesterol-lowering therapy. The full text of all publications that appeared to meet the eligibility screening process were retrieved and a second selection assessment was undertaken. Any discrepancies in the assessment and/or the decision-making of selection were resolved in discussion with another independent research investigator (MG). Two independent research investigators (JF, LMW) assessed the methodological quality of the selected full texts using the Quality Criteria Checklist for Primary Research in the American Dietetic Association Evidence Analysis Manual [50]. The Quality Criteria Checklist aided in critically appraising the quality constructs of each publication as well as to determine the relevance and validity of the selected publications. The checklist contains 10 structured validity questions including additional sub-questions specific to different research designs. The validity questions address the scientific quality, soundness, consideration of bias and confounding, the appropriateness of intervention, follow-up measures, data collection methods and statistical analyses. An overall systematic and objective rating (i.e. 'positive', 'negative' or 'neutral') was assigned to each publication. A study was deemed 'positive' if it met all priority criteria and most of the validity criteria. Priority criterions specifically address the methodology in relation to participant selection and recruitment; comparability of study groups; provision of adequate detail regarding the intervention and data collection process; use of valid and appropriate measurement tools and/or methods for study outcomes and whether potential confounders were considered. A 'Neutral' rating indicates publications that have met most of the validity criteria but have not

met one or more of the priority criterion, indicating that the study is not entirely strong, while a 'negative' rating indicates that publications have failed to meet 6 or more of the validity criteria and are therefore excluded from this review. Any discrepancies between the two independent research investigators were resolved through discussion where both parties came to an agreement.

2.4. Data extraction

Relevant data from included publications were extracted. The following data were collected: study identification (author, year, country), study design (cross-over or parallel, level of blinding), duration, sample size of each group, participant characteristics (age, gender, BMI, health status), intervention characteristics (PS dose and regime, PS type, food matrix), predominate oil used in food matrix (if margarine), baseline and post-intervention blood cholesterol outcomes (TC and LDL-C), compliance measures and study quality. Variance measures of all relevant data were also extracted. Actual PS intake calculated via compliance measures was reported for PS dose, not the intended dose. Mean and variance measures such as standard deviations, standard errors or 95% confidence intervals (CI) were collected where possible. Authors of publications were contacted to obtain missing data wherever required.

2.5. Statistical analysis

The main outcome variables for the meta-analysis were the absolute (mmol/L) and relative (%) change in TC and LDL-C concentrations due to PS intervention. TC and LDL-C concentrations were compared across the three classes of fat types. The within-trial variance measures for the absolute and relative changes in cholesterol were reported as standard errors (SE) and derived from standard deviations (SD) or 95% CI when they were not available.

When the absolute and relative changes in TC and LDL-C were not reported, they were calculated using formulae that are summarised in a systematic review conducted by Demonty et al [6].

Pooled effect sizes were calculated for the absolute and relative changes in TC and LDL-C following PS intervention using a random-effects model described by DerSimonian and Laird [51] which takes into account within and between study variation. Between study heterogeneity was quantified using the I^2 statistic, which measures the between study variation that can be attributed to heterogeneity as opposed to random variation, with the intent to assess whether studies share a common effect size. I^2 values of approximately 25%, 50% and 75% are considered to show low, moderate and high levels of heterogeneity, respectively [52].

Sensitivity analyses were conducted to assess whether any single study elicited any undue influence on the overall results. This was conducted by excluding one study at a time from the analyses and recalculating the effect size each time. Publication bias was visually assessed using a funnel plot and formally using Begg's rank correlation test [53]. The comparison between the mean reductions in TC and LDL between fat groups was compared using confidence intervals for the difference between two means.

Statistical analysis was conducted using the statistical software package STATA version 13 (StataCorp. 2013 *Stata Statistical Software: Release 13:* College Station, TX: StataCorp LP). STATA has been widely used by researchers for meta-analyses including papers that are published in reputed scientific journals [54-60]. STATA is a command line-driven programmable statistical package with commands for conducting meta-analyses. In addition to the core in-built commands, several user-written add-ons have been added and documented in the Stata Technical Bulletin (STB) [61]. All data are reported as mean ± standard error of the mean (SEM). *P*-values <0.05 were considered statistically significant.

3. RESULTS

3.1. Overview of publications

A total of 2673 publications were retrieved from the database search and one publication from hand searches. Of these, 139 publications met the inclusion criteria based on the title and abstract content. After the full texts were assessed, and further exclusions applied based on exclusion criteria, 57 publications were assessed for methodological quality. During this step, given the critical nature of assessing the quality of each publication, further exclusions were made and 35 publications were subjected to data extraction. Three publications were excluded from the meta-analysis due to insufficient data available and therefore a total of 32 studies (Figure 1) published from 1993 to 2015 comprising 49 strata were included in this systematic review (Table 1) and only 42 strata were included in the final meta-analysis (Table 2). Only the first cross-over period was included for cross-over publications in order to prevent carry-over bias [62]. The only exception to this was Geelen et al. which involved a cross-over design involving two groups which served as their own control, but differed by apoE polymorphism [63]. This study was treated as a parallel study in the meta-analysis whereby the two groups were included as separate strata. The majority of included publications were positive in quality (n=28) and only four were neutral. There were no studies with a negative quality rating.

3.2. Description of publications

A total of 2157 participants were included. The majority of publications included European participants who were otherwise healthy apart from their baseline blood cholesterol levels. Most trials were double-blinded (n=27, where both study participants and investigators are unaware of group assignment) with a mixture of parallel (n=19, participants are randomised to one of two or more intervention groups) and cross-over (n=13, participants

are their own control by receiving each intervention one after the other in a random order) designs. Twenty two studies included participants who were mild-moderate hypercholesterolaemic (≥5.5 mmol/L [3]) whereas eight included normocholesterolaemic (<5.5 mmol/L [3]) and two a combination of both hyper- and normocholesterolaemic participants. Mean age ranged from 25.0 ± 1.46 to 58.0 ± 1.06 years and mean BMI ranged from 22.2 ± 0.53 to 31.0 ± 0.52 kg/m^2. Trials ranged from three to 52 weeks in duration, with the majority of trials lasting 3 or 4 weeks. PS dosage ranged from 1.5 to 4.0 g/day. Baseline mean TC ranged from 4.97 ± 0.12 to 7.69 ± 0.16 mmol/L and mean LDL-C ranged from 2.81 ± 0.24 to 5.41 ± 0.15 mmol/L (Table 2). In the majority of strata included in the meta-analysis, the fat type comprised margarine (n=32) followed by yoghurt (n=4), cheese (n=3), butter (n=2) and milk (n=1) (Table 3). Twenty three strata were RC fat type, nine were SS and ten were D.

3.3. Effect of fat type on the phytosterol-lowering of blood cholesterol levels

The absolute net change in TC reported in studies ranged from -0.73 (95% CI: -1.08, -0.38) to -0.15 (95% CI:-0.46, 0.16) and LDL-C from -0.65 (95% CI: -1.03, -0.27) to -0.05 (95% CI: -0.91, 0.81). The relative net change in TC ranged from -13.0% (95% CI: -30.0, 5.1) to -4.4% (95% CI: -14.2, 5.0) and LDL-C from -26.0% (95% CI: -40.0, -12.0) to -5.6% (95% CI:-11.2, 0.6).

The overall pooled mean TC reduction was -0.38 mmol/L (95% CI: -0.42, -0.33; p<0.001) and the relative reduction -6.4% (95% CI: -7.3, -5.5; p<0.001) (Table 4). The overall pooled LDL-C reduction was -0.34 mmol/L (95% CI: -0.41, -0.28; p<0.001) and the relative reduction -9.3% (95% CI: -10.4, -8.2; p<0.001). Between-trial heterogeneity, assessed using the I^2 statistic was not statistically significant for the absolute or the relative changes in TC and LDL-C (p>0.05).

By fat group, overall TC reduced by -0.39 mmol/L (95% CI: -0.49, -0.30; I^2 = 0%, P = 0.62), -0.38 mmol/L (95% CI: -0.44, -0.33; I^2 = 0%, P = 0.86), and -0.32 mmol/L (95% CI: -0.43, -0.21; I^2 = 48%, P = 0.06) in the D, RC and SS groups respectively. All reductions were statistically significant (p<0.01). LDL-C reduced by -0.36 mmol/L (95% CI: -0.43, -0.29; I^2 = 0%, P = 0.48;), -0.38 mmol/L (95% CI: -0.43, -0.34; I^2 = 0%, P = 0.85), and -0.28 mmol/L (95% CI: -0.35, -0.22; I^2 = 25%, P = 0.23) in the D, RC and SS groups respectively. Reductions in LDL-C for each fat group were statistically significant (p<0.01).

The absolute mean change in LDL-C induced by the RC spreads was significantly greater compared to the SS spreads by - 0.101 mmol/L (95% CI: -0.887, - 0.097; p = 0.01). Other comparisons across fat types were not statistically significant.

Low to moderate between-trial heterogeneity was evident in the SS group only for the TC measure (I^2 = 48%, P = 0.06). Subgroup analysis was conducted to investigate this borderline heterogeneity using predefined criteria known to influence circulating cholesterol levels such as PS dosage, baseline LDL-C concentrations and BMI. Categorisation of PS dose into the following categories: <2.0 g/d; 2.0-2.4 g/d; 2.5-2.9 g/d and >3.0 g/d, demonstrated that a dose of 2.5-2.9 g/d had the greatest effect on TC (-0.57; 95% CI: -0.73, -0.40), whereas studies were more similar in terms of the effect size within the remaining PS dosages <2.0 g/d (-0.31; 95% CI: -0.40, -0.21), 2.0-2.4g/d (-0.19; 95% CI: -0.31, -0.08) and >3.0 g/d (-0.26; 95% CI: -0.46, -0.07). This is to be expected given the known dose-response relationship between PS and cholesterol-reduction. The range of PS dose across the trials for the TC measure appears to be accounting for some of the heterogeneity. Baseline LDL-C was categorised according to ATPIII [64] and does not appear to explain the observed heterogeneity given similar mean effect sizes across categories. Only one category (borderline high LDL-C) demonstrated significantly large heterogeneous effect sizes. Similarly, BMI does not appear to influence the

heterogeneity, given similar effect sizes across the BMI categories (ES ranging from -0.31; 95% CI: -0.41, -0.21 to ES -0.45; 95% CI: -1.45, 0.55).

4. DISCUSSION

This systematic review and meta-analysis has shown that PS-enriched spreads with RC as a carrier fat is superior for lowering LDL-C compared to SS. The PS-fortified products included in this review reflect those that are voluntary fortified in Australia, that is, fat spreads and dairy products e.g. low-fat milk. As expected, PS with all carrier fats significantly reduced TC and LDL-C independently, however, when compared against each other, only the RC fat type was found to reduce LDL-C significantly more than the SS group. It is noteworthy that compared to the RC and SS groups, the D group contains a diverse range of products with differing fat content; including butter (~80%), cheese (≤17%), yoghurt (≤2%) and low-fat milk (≤1.8%). Moreover, compared to the SS and RC group, the D group is complex since the level of SFA varies greatly, making it less of a homogenous group to include in the comparison against RC and SS. Therefore, plasma cholesterol reduction in the D group may have been due to a combination of factors including PS and the meal containing low fat and low SFA. It is also noteworthy that the greater heterogeneity due to varying fat content in the D group resulted in a larger confidence interval and consequently the difference did not reach a significant level compared to SS or RC groups. Given the diversity of the D group studies, the role of dairy fat in modulating the cholesterol-lowering effects of PS cannot be clearly established in the present analysis, however, the fat content of SS- and RC-containing PS products was comparable, providing a valid rationale for comparing their hypocholesterolaemic effects. The addition of PS to dairy products may be acting synergistically to improve the efficacy of their overall cholesterol-lowering ability.

Findings of this review are consistent with current scientific literature and health claims associated with PS induced cholesterol reduction [6, 65]. The overall pooled reduction in

LDL-C (both absolute and relative change) reported in this meta-analysis is similar to the findings of a previous systematic review and meta-analysis conducted by Demonty et al [6], involving analysis of 84 trials including 141 strata. Demonty et al reported a similar pooled reduction in LDL-C; -0.34 mmol/L (95% CI: -0.36, -0.31) and a relative reduction of 8.8% (95% CI: -9.4, -8.3) [6], which is comparable to the LDL-C reduction reported in the current meta-analysis.

Indeed factors that may influence the efficacy of cholesterol-lowering by PS must not be overlooked. The proportion of females and males was similar in all three groups, with less than half of the population comprising males. The age of participants was similar between the RC and D group, however, the mean age of the SS group was approximately 10 years higher. Studies have shown no direct influence of age on the efficacy of PS, however, the direct relationship between increasing age and increasing LDL-C concentrations is well established [6, 66, 67]. Since it has been shown that higher baseline cholesterol levels result in greater absolute reductions in cholesterol [6, 68], it is important to consider whether this may be influencing the findings of this review. All publications included in the meta-analysis involved participants with elevated LDL-C of >2.00 mmol/L [69]. The SS fat type had participants with the highest baseline TC and LDL-C levels, followed by D and lastly RC. The majority of publications (n=22) included participants with high TC concentrations at baseline i.e. ≥5.50 mmol/L [3]. In all three groups, the proportion of publications with baseline cholesterol concentrations >5.00 mmol/L was similar. Each fat type contained one publication including baseline TC concentrations below 5.00 mmol/L. Although the number of total publications included per fat type are not evenly distributed in this review, the significantly greater reduction in LDL-C observed with the RC group compared to the SS group is likely to be independent of baseline cholesterol levels. Most of the studies assessed background dietary intake via three- or seven-day food records with or without validation by

a Dietitian. Majority reported no change in dietary intake (including fat) pre- and post-intervention period, therefore it is unlikely that the background diet is influencing the efficacy of cholesterol-lowering induced by PS. Both sterol and stanol derived PS were included in the publications, however, it is unlikely that these are influencing the findings of this review given a meta-analysis on the continuous dose-response relationship of the LDL-C-lowering effect of PS showed that the PS type (i.e. sterols vs stanols) did not significantly affect the dose-dependent LDL-C-lowering efficacy of PS [6].

Previous studies have explored the effects of diets and/or oils rich in MUFA, PUFA and SFA on circulating cholesterol concentrations, independent of PS. The mechanisms by which PS interact with different dietary fats to influence circulating LDL-C remain to be established. The structural similarity between PS and cholesterol allows PS to compete with dietary cholesterol for intestinal absorption, however, fat plays a major role in the solubilisation of PS for micelle formation. Although the fat content of RC and SS spreads are similar, the fatty acid composition vary greatly with RC providing higher amounts of MUFA (~61%, namely oleic acid), n-3PUFA (~11%, ALA), reasonable amounts of n-6PUFA (~21%) but low levels of SFA (~7%) [32, 48]. While SS contains noticeably higher levels of n-6PUFA (~60-70%) and SFA (~13%) and relatively smaller amounts of MUFA (~20-30%) and n-3PUFA (0.5-5%) [48]. It is well known that dietary fatty acids affect circulating cholesterol levels differently, independent of PS [30-32, 43, 70, 71]. Although not previously examined, it is likely that the fatty acid composition of carrier fats may influence the degree of cholesterol displacement from the micelle, accounting for the differences in their cholesterol-lowering potential. The fat profile of RC spreads could facilitate the delivery and incorporation of PS into the micelle, thereby enhancing PS affinity for solubilisation into the micelle and heightening cholesterol displacement and overall reduction in circulating concentrations [4]. This could possibly explain the observations reported in this meta-

analysis, however, mechanisms by which different dietary fats may influence micellar formation and cholesterol displacement by PS, merits further examination.

Minimal evidence from human, animal and *in vitro* studies suggest PS may elicit a regulatory role in LDL-C metabolism. A four-week study in polygenic hypercholesterolaemic individuals administered 1.6 g/day of PS in yoghurt observed a near 10% significant increase in LDL-r affinity as well as a marked decrease (18%) in CD36 expression in addition to the expected significant decrease in plasma LDL-C (-4.3%) [72]. This observation reflects potential additional anti-atherogenic properties of PS. The potential influence of PS on LDL-r affinity could provide a complementary and/or synergistic effect between PS and carrier fats used in PS enriched fat spreads, given the upregulation of LDL-r activity elicited by PUFAs. Experimental studies are yet to report any effect of PS on LDL-r activity. Animal and human *in vitro* studies administered PS have reported increased activity of hepatic HMG-CoA reductase as well as the inhibition of NPC1L1 expression in enterocytes via reduction in their mRNA levels [73, 74]. These observations provide insight into other potential targets for optimising PS efficacy for cholesterol-lowering and overall anti-atherogenic outcomes.

The present systematic review and meta-analysis is the first to investigate whether the carrier fat in PS fortified products is a determinant of the overall hypocholesterolaemic effects of PS. This review provides an in-depth understanding of how circulating cholesterol levels may be optimised by using PS in combination with different dietary fats. A key limitation worthy of note was that several publications were excluded due to insufficient data for example type of fat used in PS fortified product and outcome data to calculate absolute and/or relative change when this was not provided. Several authors were unable to provide further information, clarification and/or did not respond to our queries, thus limiting the inclusion of additional publications. The authors of the current review decided that to assume the base fat of unidentified PS investigational products used would weaken the validity and

transparency of the results. Therefore publications were only included when the base fat could be clearly classified either through a clear description of the PS fortified product stating the type of predominant fat or if the authors reported the fatty acid profile of the PS fortified product. Previous studies have investigated the effect of using a 'dairy' product with PS, however, the fatty acid profile is not consistent with that of a true dairy-based product. Commonly the 'dairy' products used in these studies contained added vegetable fats, often in amounts that override the typical SFA composition of a regular commercial low-fat milk based on cow's milk. Therefore, in this meta-analysis we have only included dairy fat-based product studies which have a nutritional profile representative of commercial dairy milks.

Visual inspection of funnel plots and statistical assessment [53] indicated non-significant results and thus no evidence of publication bias for all fat groups and outcomes ($p>0.1$), except for relative change in LDL-C for the SS group only ($p=0.019$). Based on the relative change calculations, this could potentially result in a reduction in the effectiveness of SS in lowering LDL-C. It should be noted, however, that when comparing absolute change scores, the superiority of RC over SS was demonstrated for LDL-C.

If the effect of SS on relative changes in LDL-C is overestimated due to publication bias, this could potentially enhance the effect of RC relative to the SS group. Due to the relatively smaller number of trials available for the SS group, a trim and fill analysis to estimate the publication bias adjusted effect for relative change in LDL-C in the SS group was unable to be conducted, however, this is noted as a potential limitation in the present study. The present systematic review underpins the potential mechanisms by which PS and dietary fats influence cholesterol levels and how circulating levels might be optimised by using PS in combination with different dietary fats.

5. CONCLUSION

To the best of our knowledge, this systematic review and meta-analysis is the first to demonstrate that the carrier fat used in PS-fortified foods is a determinant of the cholesterol-lowering potential of PS. We report novel findings such that the carrier fat of common PS fortified foods does appear to modulate the cholesterol-lowering potential of PS. Furthermore, we have demonstrated that RC appear to be the superior carrier fat for optimum PS functionality. In addition to the cholesterol-lowering potential of PS fortified RC fat spreads, the health benefits of MUFA and n-3PUFA (which are the predominant fats found in RC fat spreads) support RC as an ideal carrier fat for PS, providing additional cardio-protective action to improve the lipid profile of atherogenic LDL-C by lowering its oxidation susceptibility [75-79]. Our systematic review and meta-analysis provides the first evidence that RC based PS spreads may offer dual benefit for greater cholesterol reduction as well as to facilitate the role of PS for cholesterol-lowering in hyperchoelsterolaemic individuals. The findings of this review suggest the importance of considering the fat composition used for PS fortified products in order to yield optimum cholesterol-lowering outcomes in individuals with hypercholesterolaemia.

6. ACKNOWLEDGEMENTS

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7. REFERENCES

1. Organisation WH. Cardiovascular diseases (CVDs) [Internet]. 2015 [updated January 2015; cited 2015 19 August]. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/.
2. Bloom DE, Cafiero ET, Jané-Llopis E, Abrahams-Gessel S, Bloom LR, Fathima S, et al. The Global Economic Burden of Noncommunicable Diseases. Geneva: World Economic Forum, 2011 September. Report No.: 080911.
3. Australian Institute of Health and Welfare. High blood cholesterol. Cat. no. AUS 156. ed. Canberra: Australian Institute of Health and Welfare; 2012-2013.
4. De Smet E, Mensink RP, Plat J. Effects of plant sterols and stanols on intestinal cholesterol metabolism: suggested mechanisms from past to present. Mol Nutr Food Res. 2012;56(7):1058-72.
5. Joyce T. Stanols and Sterols. The British Dietetic Association, 2015.
6. Demonty I, Ras RT, van der Knaap HC, Duchateau GS, Meijer L, Zock PL, et al. Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr. 2009;139(2):271-84.
7. Gylling H, Plat J, Turley S, Grinsberg HN, Ellegard L, Jessup W, et al. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. Atheroscler. 2014;232.
8. Patel MD, Thompson PD. Phytosterols and vascular disease. Atheroscler. 2006;186:12-9.
9. Micallef MA, Garg ML. Beyond blood lipids: phytosterols, statins and omega-3 polyunsaturated fatty acid therapy for hyperlipidemia. J Nutr Biochem. 2009;20(12):927-39.
10. Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, Parsons WE. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res [Internet]. 2000; 41(5):[697-705 pp.].
11. Garti N, Avrahami M, Aserin A. Improved solubilization of celecoxib in U-type nonionic microemulsions and their structural transitions with progressive aqueous dilution. J Colloid Interface Sci. 2006;299(1):352-65.
12. Ling WH, Jones PJ. Dietary phytosterols: a review of metabolism, benefits and side effects. Life Sci. 1995;57(3):195-206.
13. Plat J, Mensink RP. Plant stanol and sterol esters in the control of blood cholesterol levels: Mechanism and safety aspects. Am J Cardiol. 2005;96(1 SUPPL.):15D-22D.
14. Peterson DW. Effect of soybean sterols in the diet on plasma and liver cholesterol in chicks. Proc Soc Exp Biol Med. 1951;78:671-7.
15. National Heart Foundation of Australia. Phytosterol/Stanol enriched foods. National Australian Heart Foundation, 2007-2009 December. Report No.: PRO-095.
16. Ras RT, Geleijnse JM, Trautwein EA. LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: a meta-analysis of randomised controlled studies. British Journal of Nutrition. 2014;112(2):214-9.
17. LaRosa JC. Low-density lipoprotein cholesterol reduction: the end is more important than the means. Am J Cardiol. 2007;100(2):240-2.
18. Blair SN, Capuzzi DM, Gottlieb SO, Nguyen T, Morgan JM, Cater NB. Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am J Cardiol. 2000;86(1):46-52.
19. Neil HA, Meijer GW, Roe LS. Randomised controlled trial of use by hypercholesterolaemic patients of a vegetable oil sterol-enriched fat spread. Atheroscler. 2001;156(2):329-37.

20. Castro Cabezas M, de Vries JH, Van Oostrom AJ, Iestra J, van Staveren WA. Effects of a stanol-enriched diet on plasma cholesterol and triglycerides in patients treated with statins. J Am Diet Assoc. 2006;106(10):1564-9.
21. Nicholls SJ, Brandrup-Wognsen G, Palmer M, Barter PJ. Meta-analysis of comparative efficacy of increasing dose of Atorvastatin versus Rosuvastatin versus Simvastatin on lowering levels of atherogenic lipids (from VOYAGER). Am J Cardiol. 2010;105(1):69-76.
22. Noakes M, Clifton P, Ntanios F, Shrapnel W, Record I, McInerney J. An increase in dietary carotenoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations. Am J Clin Nutr. 2002;75(1):79-86.
23. Fernandez ML, West KL. Mechanisms by which Dietary Fatty Acids Modulate Plasma Lipids. J Nutr. 2005;135:2075-78.
24. Dias CB, Garg R, Wood LG, Garg ML. Saturated fat consumption may not be the main cause of increased blood lipid levels. Med Hypotheses. 2014;82(2):187-95.
25. Hayes KC. Saturated fats and blood lipids: new slant on an old story. Can J Cardiol. 1995;11(Suppl G):39G-46G.
26. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, et al. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr. 1999;70:1001-8.
27. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77:1146-55.
28. Hegsted DM, Mcgandy RB, Myers L, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. . Am J Clin Nutr. 1965;17:281-95.
29. Hunter JE, Zhang J, Kris-Etherton PM. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. The American journal of clinical nutrition. 2010;91(1):46-63.
30. Gillingham LG, Gustafson JA, Han SY, Jassal DS, Jones PJ. High-oleic rapeseed (canola) and flaxseed oils modulate serum lipids and inflammatory biomarkers in hypercholesterolaemic subjects. Br J Nutr. 2011;105(3):417-27.
31. Negele L, Schneider B, Ristl R, Stulnig TM, Willfort-Ehringer A, Helk O, et al. Effect of a low-fat diet enriched either with rapeseed oil or sunflower oil on plasma lipoproteins in children and adolescents with familial hypercholesterolaemia. Results of a pilot study. Eur J Clin Nutr. 2015;69(3):337-43.
32. Lin L, Allemekinders H, Dansby A, Campbell L, Durance-Tod S, Berger A, et al. Evidence of health benefits of canola oil. Nutr Rev. 2013;71(6):370-85.
33. Gustafson I, Vessby B, Ohrvall M, Nydahl M. A diet rich in monounsaturated rapeseed oil reduces the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemic subjects. Am J Clin Nutr. 1994;59:667-74.
34. Valsta LM, Jauhiainen M, Aro A, Katan MB, Mutanen M. Effects of a monounsaturated rapeseed oil and a polyunsaturated sunflower oil diet on lipoprotein levels in humans. Arterioscler Thromb. 1992;12:50-7.
35. Dietschy JM. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. J Nutr. 1998;128(2 Suppl):444S-8S.
36. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients. Curr Atheroscler Rep. 2010;12(6):384-90.
37. Hayes KC, Khosla P, Hajri T, Pronczuk A. Saturated fatty acids and LDL receptor modualtion in humans and monkeys. Prostaglandins Leukot Essent Fatty Acids. 1997;57(4 & 5):411-18.

38. Daumerie CM, Woollett LA, Dietschy JM. Fatty acids regulate hepatic low density lipoprotein receptor activity through redistribution of intracellular cholesterol pools. Proc Natl Acad Sci. 1992;89(22):10797-801.
39. Spady DK, Woollett LA, Dietschy JM. Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. Annu Rev Nutr 1993;13:355-81.
40. Rumsey SC, Galeano NF, Lipschitz B, Deckelbaum RJ. Oleate and other long chain fatty acids stimulate low density lipoprotein receptor activity by enhancing acyl coenzyme A:cholesterol acyltransferase activity and altering intracellular regulatory cholesterol pools in cultured cells. J Biol Chem. 1995;270:10008-16.
41. Degirolamo C, Shelness GS, Rudel LL. LDL cholesteryl oleate as a predictor for atherosclerosis: evidence from human and animal studies on dietary fat. J Lipid Res. 2009;50(Suppl):S434-9.
42. Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res. 1985;26:194-202.
43. Kris-Etherton P. Monounsaturated Fatty Acids and Risk of Cardiovascular Disease. Circulation. 1999;100:1253-8.
44. Hargrove RL, Etherton TD, Pearson TA, Harrison EH, Kris-Etherton P. Low fat and high monounsaturated fat diets decrease human low density lipoprotein oxidative susceptibility in vitro. J Nutr. 2001;131:1758-63.
45. Mustad VA, Ellsworth JL, Cooper AD, Kris-Etherton PM, Etherton TD. Dietary linoleic acid increases and palmitic acid decreases hepatic LDL receptor protein and mRNA abundance in young pigs. J Lipid Res. 1996;37:2310-23.
46. Tobin KA, Steineger HH, Alberti S, Spydevold O, Auwerx J, Gustafsson JA, et al. Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor-alpha. Mol Endrocrinol. 2000;14(5):741-52.
47. Illingworth DR, Schmidt EB. The influence of dietary n-3 fatty acids on plasma lipids and lipoproteins. Ann NY Acad Sci. 1993;676:60-9.
48. Dubios V, Breton S, Linder M, Fanni J, Parmentier M. Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. Eur J Lipid Sci Technol. 2007;109:710-32.
49. Rifai N, Warnick GR, Dominiczak MH. Handbook of Lipoprotein Testing. 2nd ed: AACC Press; 2001 March 1. 819 p.
50. Dietetics. AoNa. Evidence Analysis Manual: Steps in the Academy Evidence Analaysis Process. In: Development RaSB, editor. 2012. p. 1-111.
51. DerSimonioan R, Laird N. Meta-analysis in clinical trials. Controlled Clin Trials. 1986;7(3):177-88.
52. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Br Med J. 2003;327(7414):557-60.
53. Begg CB, Mazumdar M. Operating Characteristics of a Rank Correlation Test for Publication Bias. Biometrics. 1994;50(4):1088-101.
54. Genser B, Silbernagel G, De Backer G, Bruckert E, Carmena R, Chapman MJ, et al. Plant sterols and cardiovascular disease: a systematic review and meta-analysis. Eur Heart J. 2012;33(4):444-51.
55. Threapleton DE, Greenwood DC, Evans CE, Cleghorn CL, Nykjaer C, Woodhead C, et al. Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. BMJ. 2013;347:f6879.
56. Wang F, Zheng J, Yang B, Jiang J, Fu Y, Li D. Effects of Vegetarian Diets on Blood Lipids: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Am Heart Assoc. 2015;4(10):e002408.

57. Wu L, Wang Z, Zhu J, Murad AL, Prokop LJ, Murad MH. Nut consumption and risk of cancer and type 2 diabetes: a systematic review and meta-analysis. Nutr Rev. 2015;73(7):409-25.
58. Zhou D, Yu H, He F, Reilly KH, Zhang J, Li S, et al. Nut consumption in relation to cardiovascular disease risk and type 2 diabetes: a systematic review and meta-analysis of prospective studies. Am J Clin Nutr. 2014;100(1):270-7.
59. Liu ZM, Chen YM, Ho SC. Effects of soy intake on glycemic control: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2011;93(5):1092-101.
60. Mohammadifard N, Salehi-Abargouei A, Salas-Salvado J, Guasch-Ferre M, Humphries K, Sarrafzadegan N. The effect of tree nut, peanut, and soy nut consumption on blood pressure: a systematic review and meta-analysis of randomized controlled clinical trials. Am J Clin Nutr. 2015;101(5):966-82.
61. The Stata Journal: StataCorp LP; 2001-2016. Available from: http://www.stata-journal.com.
62. Curtin F, Elbourne D, Altman DG. Meta-analysis combining parallel and cross-over clinical trials. III: The issue of carry-over. Stat Med. 2002;21:2161-73.
63. Geelen A, Zock PL, de Vries JH, Katan MB. Apolipoprotein E polymorphism and serum lipid response to plant sterols in humans. E J Clin Invest. 2002;32(10):738-42.
64. Cleeman JI, Grundy SM, Becker D, Clark LT, Cooper RS, Denke MA, et al. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). J Am Med Assoc. 2001;285:2486-97.
65. Clifton PM, Noakes M, Sullivan D, Erichsen N, Ross D, Annison G, et al. Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. E J Clin Nutr. 2004;58(3):503-9.
66. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R, et al. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clinic proceedings. 2003;78(8):965-78.
67. Law M. Plant sterol and stanol margarines and health. B Med J. 2000;320:861-4.
68. Musa-Veloso K, Poon TH, Elliot JA, Chung C. A comparison of the LDL-cholesterol lowering efficacy of plant stanols and plant sterols over a continuous dose range: Results of a meta-analysis of randomized, placebo-controlled trials. Prostaglandins Leukot Essent Fatty Acids [Internet]. 2011; 85(1):[9-28 pp.].
69. Cholesterol: Better Health Channel; [updated February 2014; cited 2015 9 Dec]. Available from: https://www.betterhealth.vic.gov.au/health/conditionsandtreatments/cholesterol.
70. Grundy SM. What is the desirable ratio of saturated, polyunsaturated, and monounsaturated fatty acids in the diet? Am J Clin Nutr. 1997;66(Suppl):988S-90S.
71. Gardner CD, Kraemer HC. Monounsaturated versus polyunsaturated dietary fat and serum lipids: a meta-analysis. Arterioscler Thromb Vasc Biol. 1995;15:1917-27.
72. Ruiu G, Pinach S, Veglia F, Gambino R, Marena S, Uberti B, et al. Phytosterol-enriched yogurt increases LDL affinity and reduces CD36 expression in polygenic hypercholesterolemia. Lipids. 2009;44(2):153-60.
73. Jesch ED, Seo JM, Carr TP, Lee JY. Sitosterol reduces messenger RNA and protein expression levels of Niemann-Pick C1-like 1 in FHs 74 Int cells. Nutr Res. 2009;29(12):859-66.
74. Moghadasian MH, Nguyen LB, Shefer S, Salen G, Batta AK, Frohlich JJ. Hepatic cholesterol and bile acid synthesis, low-density lipoprotein receptor function, and plasma and fecal sterol levels in mice: effects of apolipoprotein E deficiency and probucol or phytosterol treatment. Metabolism. 2001;50(6):708-14.

75. Corby J. Fatty acid composition and the oxidation of low-density lipoproteins. Biochem Med Metab Biol. 1993;49:25-35.
76. Turpeinen AM, Alfthan G, Valsta L, Hietanen E, Salonen JT, Schunk H, et al. Plasma and lipoprotein lipid peroxidation in humans on sunflower and rapeseed oil diets. Lipids. 1995;30(6):485-92.
77. Palomaki A, Pohjantahti-Maaroos H, Wallenius M, Kankkunen P, Aro H, Husgafvel S, et al. Effects of dietary cold-pressed turnip rapeseed oil and butter on serum lipids, oxidized LDL and arterial elasticity in men with metabolic syndrome. Lipids Health Dis. 2010;9:137.
78. Nielsen NS, Pedersen A, Sandström B, Marckmann P, Høy C-E. Different effects of diets rich in olive oil, rapeseed oil and sunflower-seed oil on postprandial lipid and lipoprotein concentrations and on lipoprotein oxidation susceptibility. Br J Nutr. 2002;87(05):489-99.
79. Egert S, Kratz M, Kannenberg F, Fobker M, Wahrburg U. Effects of high-fat and low-fat diets rich in monounsaturated fatty acids on serum lipids, LDL size and indices of lipid peroxidation in healthy non-obese men and women when consumed under controlled conditions. Eur J Nutr. 2011;50(1):71-9.
80. Alhassan S, Reese KA, Mahurin J, Plaisance EP, Hilson BD, Garner JC, et al. Blood lipid responses to plant stanol ester supplementation and aerobic exercise training. Metab Clin Exp [Internet]. 2006; 55(4):[541-9 pp.].
81. Baumgartner S, Mensink RP, Husche C, Lutjohann D, Plat J. Effects of plant sterol- or stanol-enriched margarine on fasting plasma oxyphytosterol concentrations in healthy subjects. Atheroscler. 2013;227(2):414-9.
82. Blomqvist SM, Jauhiainen M, van Tol A, Hyvonen M, Torstila I, Vanhanen H, et al. Effect of sitostanol ester on composition and size distribution of low- and high-density lipoprotein. Nutr Metab Cardiovasc Dis. 1993;3:158-64.
83. Buyuktuncer Z, Fisunoglu M, Guven GS, Unal S, Besler HT. The cholesterol lowering efficacy of plant stanol ester yoghurt in a Turkish population: A double-blind, placebo-controlled trial. Lipids Health Dis [Internet]. 2013; 12(1).
84. Charest A, Vanstone C, St-Onge MP, Parson W, Jones PJ, Lamarche B. Phytosterols in nonfat and low-fat beverages have no impact on the LDL size phenotype. E J Clin Nutr. 2005;59(6):801-4.
85. Christiansen LI, Lahteenmaki PL, Mannelin MR, Seppanen-Laakso TE, Hiltunen RV, Yliruusi JK. Cholesterol-lowering effect of spreads enriched with microcrystalline plant sterols in hypercholesterolemic subjects. E J Nutr. 2001;40(2):66-73.
86. Gagliardi AC, Maranhao RC, de Sousa HP, Schaefer EJ, Santos RD. Effects of margarines and butter consumption on lipid profiles, inflammation markers and lipid transfer to HDL particles in free-living subjects with the metabolic syndrome. E J Clin Nutr. 2010;64(10):1141-9.
87. Gylling H, Radhakrishnan R, Miettinen TA. Reduction of serum cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine: women and dietary sitostanol. Circulation. 1997;96(12):4226-31.
88. Gylling H, Halonen J, Lindholm H, Konttinen J, Simonen P, Nissinen MJ, et al. The effects of plant stanol ester consumption on arterial stiffness and endothelial function in adults: A randomised controlled clinical trial. BMC Cardiovasc Disord. 2013;13.
89. Hallikainen MA, Uusitupa MI. Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am J Clin Nutr. 1999;69(3):403-10.
90. Hallikainen MA, Sarkkinen ES, Gylling H, Erkkila AT, Uusitupa MI. Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum

cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. E J Clin Nutr. 2000;54(9):715-25.
91. Hallikainen MA, Sarkkinen ES, Uusitupa MI. Plant stanol esters affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependent manner. J Nutr. 2000;130(4):767-76.
92. Hallikainen M, Lyyra-Laitinen T, Laitinen T, Agren JJ, Pihlajamaki J, Rauramaa R, et al. Endothelial function in hypercholesterolemic subjects: Effects of plant stanol and sterol esters. Atherosclerosis. 2006;188(2):425-32.
93. Hallikainen M, Lyyra-Laitinen T, Laitinen T, Moilanen L, Miettinen TA, Gylling H. Effects of plant stanol esters on serum cholesterol concentrations, relative markers of cholesterol metabolism and endothelial function in type 1 diabetes. Atheroscler. 2008;199(2):432-9.
94. Hendriks HF, Weststrate JA, van Vliet T, Meijer GW. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. E J Clin Nutr. 1999;53(4):319-27.
95. Hyun YJ, Oh YK, Joo BK, Lee JH, Jang Y, Liponkoski L, et al. Plant stanol esters in low-fat yogurt reduces total and low-density lipoprotein cholesterol and low-density lipoprotein oxidation in normocholesterolemic and mildly hypercholesterolemic subjects. Nutr Res. 2005;25(8):743-53.
96. Jauhiainen T, Salo P, Niittynen L, Poussa T, Korpela R. Effects of low-fat hard cheese enriched with plant stanol esters on serum lipids and apolipoprotein B in mildly hypercholesterolaemic subjects. E J Clin Nutr. 2006;60(11):1253-7.
97. Korpela R, Tuomilehto J, Hogstrom P, Seppo L, Piironen V, Salo-Vaananen P, et al. Safety aspects and cholesterol-lowering efficacy of low fat dairy products containing plant sterols. E J Clin Nutr. 2006;60(5):633-42.
98. Lottenberg AM, Nunes VS, Nakandakare ER, Neves M, Bernik M, Lagrost L, et al. The human cholesteryl ester transfer protein I405V polymorphism is associated with plasma cholesterol concentration and its reduction by dietary phytosterol esters. J Nutr. 2003;133(6):1800-5.
99. Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med. 1995;333(20):1308-12.
100. Nigon F, Serfaty-Lacrosniere C, Beucler I, Chauvois D, Neveu C, Giral P, et al. Plant sterol-enriched margarine lowers plasma LDL in hyperlipidemic subjects with low cholesterol intake: effect of fibrate treatment. Clin Chem Lab Med. 2001;39(7):634-40.
101. Plat J, Mensink RP. Vegetable oil based versus wood based stanol ester mixtures: effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. Atherosclerosis. 2000;148(1):101-12.
102. Ras RT, Demonty I, Zebregs YE, Quadt JF, Olsson J, Trautwein EA. Low doses of eicosapentaenoic acid and docosahexaenoic acid from fish oil dose-dependently decrease serum triglyceride concentrations in the presence of plant sterols in hypercholesterolemic men and women. J Nutr. 2014;144(10):1564-70.
103. Ras RT, Fuchs D, Koppenol WP, Garczarek U, Greyling A, Keicher C, et al. The effect of a low -fat spread with added plant sterols on vascular function markers: results of the Investigating Vascular Function Effects of Plant Sterols (INVEST) study. Am J Clin Nutr. 2015;101(4):733-41.
104. Simons LA. Additive effect of plant sterol-ester margarine and cerivastatin in lowering low-density lipoprotein cholesterol in primary hypercholesterolemia. Am J Cardiol. 2002;90(7):737-40.

105. Theuwissen E, Plat J, Van Der Kallen CJ, Van Greevenbroek MM, Mensink RP. Plant stanol supplementation decreases serum triacylglycerols in subjects with overt hypertriglyceridemia. Lipids. 2009;44(12):1131-40.
106. Turpeinen AM, Ikonen M, Kivimäki AS, Kautiainen H, Vapaatalo H, Korpela R. A spread containing bioactive milk peptides Ile-Pro-Pro and Val-Pro-Pro, and plant sterols has antihypertensive and cholesterol-lowering effects. Food Funct [Internet]. 2012; 3(6):[621-7 pp.].
107. Vanstone CA, Raeini-Sarjaz M, Parsons WE, Jones PJ. Unesterified plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. Am J Clin Nutr. 2002;76(6):1272-8.
108. Vasquez-Trespalacios EM, Romero-Palacio J. Efficacy of yogurt drink with added plant stanol esters (Benecol, Colanta) in reducing total and LDL cholesterol in subjects with moderate hypercholesterolemia: A randomized placebo-controlled crossover trial NCT01461798. Lipids Health Dis [Internet]. 2014; 13(1).
109. Vissers MN, Zock PL, Meijer GW, Katan MB. Effect of plant sterols from rice bran oil and triterpene alcohols from sheanut oil on serum lipoprotein concentrations in humans. Am J Clin Nutr. 2000;72(6):1510-5.
110. Gylling H, Hallikainen M, Raitakari OT, Laakso M, Vartiainen E, Salo P, et al. Long-term consumption of plant stanol and sterol esters, vascular function and genetic regulation. B J Nutr. 2009;101(11):1688-95.
111. Hendriks HF, Brink EJ, Meijer GW, Princen HM, Ntanios FY. Safety of long-term consumption of plant sterol esters-enriched spread. E J Clin Nutr. 2003;57(5):681-92.
112. Charest A, Desroches S, Vanstone CA, Jones PJ, Lamarche B. Unesterified plant sterols and stanols do not affect LDL electrophoretic characteristics in hypercholesterolemic subjects. J Nutr. 2004;134(3):592-5.

8. TABLES

Table 1

Inclusion and exclusion criteria for the selection of publications

Inclusion	Exclusion
RCT	Observational studies (i.e. cross-sectional, longitudinal), case control, quasi-randomised control trials, non-randomised control trials, any other that was not an RCT
Humans	
Adults (aged ≥ 18 years)	
Blood cholesterol concentrations as primary or secondary outcomes	Children, animal, *in vivo* and *in vitro* studies
PS dosage ≥ 1.5g/day	Studies using food format other than margarine or dairy[2]
Publication published year 1990 to present	
Publications published in English	Studies where fat type could not be ascertained[3]
Predominant fat type of food product was stated[1]	Co-intervention with cholesterol-lowering therapy without PS-placebo treatment arm[4]
	Insufficient relevant blood cholesterol data
	PS consumed for ≤ 3 weeks
	Non-English

[1] Publications which reported 40% or greater of total fatty acids as linoleic acid were included in the SS group (n=3).

[2] One publication was removed because it the intervention was a dual food format including both margarine and milk enriched with PS.

[2] Fat type was not ascertained when the authors of the publication had not specified the fat type the product was based on and when authors could not confirm the predominant fat type or could not be contacted.

[3] Publications that included a PS-placebo group (i.e. PS was administered in isolation of cholesterol-lowering therapy) were included, along with the control group.

Table 2

Overview of the publications included in the systematic review and meta-analysis (n= 32)

Author, publication year, quality score[1] and reference	Location and study design	Subject Characteristics						Treatment Characteristics			
		Sample size		Mean age (y)[2]	Mean baseline BMI (kg/m²)[2]	Mean baseline TC (mmol/L)[3]	Mean baseline LDL-C (mmol/L)[3]	Food matrix	Fat type	Duration (weeks)	PS dose (g/d)
		C	T								
Alhassan et al. 2006 (+) [80]	USA, Parallel	9	17	53.0 ± (8.0)	27.0 ± (1.0)	5.23 ± (0.21)	3.32 ± (1.18)	Margarine	RC	4	3.0
Baumgartner et al. 2013 (+) [81]	Netherlands Cross-over	43		41 ± (2.74)	24.8 ± (0.43)	5.72 ± (0.17)[a]	3.53 ± (0.16)[a]	Margarine	RC	4	3.0
Blomqvist et al. 1993 (ø) [82]	Finland, Parallel	32	34	NR	NR	5.84 ± (0.13)	3.35 ± (0.12)	Margarine	RC	4	3.4
Buyuktuncer et al. 2013 (+) [83]	Netherlands, Parallel	35	35	45.5 ± (1.19)[b]	27.9 ± (0.53)[b]	6.24 ± (0.11)	4.09 ± (0.11)	Yoghurt	D	4	1.9
Charest et al. 2004 (+) [84]	Canada, Cross-over										
- Stratum 1		14		48.6 ± (1.82)	30.9 ± (1.39)	6.0 ± (0.29)	4.0 ± (0.21)	Butter	D	3	1.8
- Stratum 2						6.2 ± (0.24)	4.1 ± (0.19)	Butter	D	3	1.8
- Stratum 3						6.4 ± (0.35)	4.2 ± (0.27)	Butter	D	3	1.8
Christiansen et al. 2001 (+) [85]	Finland, Parallel										
- Stratum 1		17	19	50.7[c]	24.9 ± (0.88)	6.66 ± (0.07)[a]	4.29 ± (0.06)[a]	Margarine	RC	24	1.5
- Stratum 2			16	50.7[c]	25.2 ± (0.68)			Margarine	RC	24	3.0
Clifton et al. 2004 (+) [65]	Australia Cross-over										
- Stratum 1		58	40[d]	54 ± (1.05)	26.2 ± (0.39)	6.24 ± (0.10)[a]	4.03 ± (0.09)[a]	Milk	D	3	1.6
- Stratum 2			40[d]					Yoghurt	D	3	1.6
Gagliardi et al. 2010 (+) [86]	Brazil, Parallel	16	19	47.4 ± (1.33)	31.3 ± (0.47)	5.31 ± (0.18)[e]	3.26 ± (0.19)[e]	Margarine	SS	5	2.4
Geelan et al. 2002 (ø)	Netherlands, Cross-over										
- Stratum 1			31	26 ± (2.16)	23 ± (0.54)	5.2 ± (0.16)	–	Margarine	RC	3	3.2

- Stratum 2			57	25 ± (1.46)	23 ± (0.26)	4.6 ± (0.12)	_	Margarine	RC	3	3.2
Gylling et al. 1997 (+) [87]	Finland, Parallel	11	11	51 ± (1)	26.0 ± (0.7)	6.26 ± (0.20)	3.85 ± (0.17)	Margarine	RC	7	3.0
Gylling et al. 2013 (+) [88]	Finland, Parallel	46	46	50.9 ± (1.4)[b]	25.4 ± (0.6)[b]	5.48 ± (0.12)	3.52 ± (0.12)	Margarine	RC	24	3.0
Hallikainen et al. 1999 (+) [89]	Finland Parallel										
- Stratum 1		17	18	43.2 ± (1.93)[b]	25.6 ± (0.94)[b]	6.55 ± (0.18)	4.54 ± (0.17)	Margarine	RC	8	2.31
- Stratum 2			20	40.8 ± (2.08)[b]	24.2 ± (0.67)[b]	6.13 ± (0.18)	4.25 ± (0.19)	Margarine	RC	8	2.16
Hallikainen et al. 2000 i (+) [90]	Finland Cross-over										
- Stratum 1		34		48.8 ± (1.39)	24.9 ± (0.41)	6.24 ± (0.14)	4.43 ± (0.14)	Margarine	RC	4	2.04
- Stratum 2								Margarine	RC	4	2.01
Hallikainen et al. 2000 ii (+) [91]	Finland, Cross-over										
- Stratum 2		22		50.5 ± (2.49)	26.2 ± (0.75)	6.87 ± (0.27)[a]	4.81 ± (0.23)[a]	Margarine	RC	4	1.6
- Stratum 3								Margarine	RC	4	2.4
- Stratum 4								Margarine	RC	4	3.2
Hallikainen et al. 2006 (+) [92]	Finland, Cross-over										
- Stratum 1		37	39	50.0 ± (2.1)[b]	25.7 ± (0.6)[b]	5.52 ± (0.18)	3.31 ± (0.16)	Margarine	RC	10	1.92
- Stratum 2								Margarine	RC	10	1.97
Hallikainen et al. 2008 (+) [93]	Finland, Parallel	8	11	39.6 ± (4.2)[b]	24.5 ± (1.0)[b]	5.26 ± (0.07)	2.81 ± (0.24)	Margarine	RC	12	2.0
Hendriks et al. 1999 (+) [94]	Netherlands, Cross-over	80		37 ± (1.12)	22.8 ± (0.28)	5.10 ± (0.11)[a]	2.97 ± (0.09)[a]	Margarine	SS	3.5	1.61
- Stratum 1											
- Stratum 2								Margarine	SS	3.5	3.24
Hyun et al. 2005 (+) [95]	Korea Parallel	28	23	28.7 ± (0.7)	22.6 ± (0.4)	4.97 ± (0.12)	3.08 ± (0.12)	Yoghurt	D	4	2.0
Jauhiainen et al. 2006 (+) [96]	Finland, Parallel	34	33	43.3 ± (1.08)	NR	5.65 ± (0.09)	3.58 ± (0.09)	Cheese	D	5	2.0
Korpela et al. 2006 (+) [97]	Finland, Parallel	29	33	57.6 ± (1.00)[b]	27.1 ± (0.43)[b]	6.42 ± (0.08)[a]	4.05 ± (0.08)[a]	Hard cheese	D	6	2.0

- Stratum 1											
		28	24					Fresh cheese	D	6	2.0
- Stratum 2											
- Stratum 3		25	25					Yoghurt	D	6	2.0
Lottenberg et al. 2003 (ø) [98]	Brazil, Cross-over	30	30	55.8[c]	26.4[b]	7.0 ± (0.10)[a]	5.0 ± (0.09)[a]	Margarine	SS	4	1.68
Miettinen et al. 1995 (+) [99]	Finland, Parallel										
- Stratum 1		51	51	49 ± (2)	NR	6.01 ± (0.10)	3.96 ± (0.10)	Margarine	RC	52	1.8
- Stratum 2			51	51 ± (1)	NR	6.06 ± (0.10)	4.14 ± (0.10)	Margarine	RC	52	2.6
Nigon et al. 2001 (+)[f] [100]	France, Cross-over		34	55 ± (2.23)	22.8 ± (0.43)	6.73 ± (0.11)	4.56 ± (0.10)	Margarine	SS	8	1.6
Plat et al. 2000 (+) [101]	Netherlands, Parallel										
- Stratum 1		42	36	33 ± (2.5)[b]	22.2 ± (0.78)[b]	4.99 ± (0.12)	2.94 ± (0.12)	Margarine	RC	8	3.8
- Stratum 2			34	33 ± (2.57)[b]	23.4 ± (0.53)[b]	4.98 ± (0.16)	2.94 ± (0.15)	Margarine	RC	8	4.0
Ras et al. 2014 (+) [102]	Sweden, Parallel	62	64	57.9 ± (0.6)	25.0 ± (0.1)	6.39 ± (0.10)	3.91 ± (0.09)	Margarine	SS	4	2.5
Ras et al. 2015 (+) [103]	Germany, Parallel	105	113	53.2 ± (0.44)	25.3 ± (0.18)	5.75 ± (0.09)	3.89 ± (0.06)	Margarine	SS	12	3.0
Simons 2002 (+) [104]	Australia Parallel	38	39	58 ± (1.60)[b]	27.6 ± (0.58)[b]	7.69 ± (0.16)	5.41 ± (0.15)	Margarine	SS	4	2.0
Theuwissen et al. 2009 (+) [105]	Netherlands, Parallel	14	14	54 ± (1.51)	28 ± (0.57)	7.35 ± (0.18)	4.96 ± (0.19)	Margarine	SS	3	2.5
Turpeinen et al. 2012 (+) [106]	Finland Parallel	49	51	49 ± (0.84)[b]	28.3 ± (0.66)[b]	6.0 ± (0.10)	3.8 ± (0.08)	Margarine	RC	10	2.0
Vanstone et al. 2002 (+) [107]	Canada, Cross-over										
- Stratum 1			15	47.8 ± (1.9)	30.8 ± (1.3)	5.97 ± (0.26)	4.0 ± (0.20)	Butter	D	3	1.8
- Stratum 2						6.23 ± (0.26)	4.11 ± (0.18)	Butter	D	3	1.8
- Stratum 3						6.40 ± (0.31)	4.18 ± (0.23)	Butter	D	3	1.8
Vasquez-Trespalacious et al. 2014 (+)[g] [108]	USA, Cross-over		40	37.9 ± (1.34)	25.0 ± (0.54)	NR [g]	NR	Yoghurt	D	4	4.0

Vissers et al. 2000 (ø)[h]	Netherlands, Cross-over										
- Stratum 1		60	NR	NR	NR	NR	Margarine	SS	3	2.1	

[1] American Dietetic Association's Quality Criteria Checklist quality score given in brackets: (+) = positive, (ø) = neutral and (-) negative.

[2] Data is reported for total sample size unless otherwise specified.

[3] Mean baseline TC (mmol/L) and LDL-C (mmol/L) data are reported for treatment group only unless otherwise specified.

[a] Data reported for total sample size only.

[b] Data only reported separate for control and intervention group. Therefore value presented is for intervention group only.

[c] Measure of spread not reported.

[d] Total sample size was N = 58, however, N=58 completed control period and only 40 completed the study for the milk and yoghurt intervention group.

[e] SE calculated from the range using formula: SD = (maximum – minimum)/4. SD converted to SE using formula described in section 2.5.

[f] Only stratum 1 included (plant-sterol alone therapy). Stratum 2 excluded for fibrate and plant sterol co-therapy.

[g] Authors only included moderately hyperchoelsterolaemic individuals (5.2-7.5 mmol/L) [108].

[h] Only one strata was included as only this strata met the inclusion criteria. Authors only included normolipidaemic individuals [109].

C, Control group; T, Treatment group; RC, Rapeseed/canola group; D, Dairy group; SS, Sunflower/soybean group; NR, not reported.

Stratum refers to a single treatment group.

Table 3

Summary of absolute and relative change data for included strata (n = 42)

Fat Group	Author, publication year and reference	Blood cholesterol outcomes							
		TC (mmol/L)				LDL-C (mmol/L)			
		Net Δ (mmol/L)[1]	95%CI	Relative Δ (%)[1]	95%CI	Net Δ (mmol/L)[1]	95%CI	Relative Δ (%)[1]	95%CI
RC	Alhassan et al. 2006 [80]	-0.650	(-1.490, 0.190)	-13.0	(-30.0, 5.1)	-0.650	(-1.030, -0.270)	-20.4	(-42.0, 1.2)
	Baumgartner et al. 2013 [81]	-0.300	(-0.450, -0.150)	-5.3	(-8.4, -2.2)	-0.290	(-0.420, -0.160)	-8.1	(-13.0, -3.3)
	Blomqvist et al. 1993 [82]	-0.370	(-0.900, 0.160)	-6.0	(-16.0, 3.0)	-0.330	(-0.780, 0.120)	-10.0	(-22.0, 2.0)
	Christiansen et al. 2001 [85]								
	- Stratum 1	-0.620	(-1.030, -0.210)	-8.9	(-14.0, -3.0)	-0.470	(-0.860, -0.078)	-11.3	(-17.0, -5.0)
	- Stratum 2	-0.580	(-0.990, -0.170)	-8.3	(-13.2, -4.2)	-0.450	(-0.880, -0.020)	-10.6	(-16.0, -5.0)
	Geelan et al. 2002 [63]								
	- Stratum 1	-0.310	(-0.450, -0.170)	-6.0	(-15.0, 3.0)	-0.310	(-0.480, -0.140)	-9.9	(-18.0, -1.6)
	- Stratum 2	-0.360	(-0.440, -0.280)	-8.0	(-15.0, -0.7)	-0.340	(-0.470, -0.210)	-12.4	(-20.0, -5.0)
	Gylling et al. 1997 [87]	-0.550	(-1.350, 0.250)	-8.0	(-16.0, 0.0)	-0.530	(-0.760, -0.300)	-14.5	(-42.0, 13.0)
	Gylling et al. 2009 [110]	NR	NR			NR	NR	NR	NR
	- Stratum 1			-4.4	(-7.3, -1.5)				
	- Stratum 2			-4.2	(-7.1, 2.9)				
	Gylling et al. 2013 [88]	-0.360	(-0.580, -0.140)	-6.6	(-10.3, -2.9)	-0.350	(-0.530, -0.170)	-10.2	(-19.0, -4.1)
	Hallikainen et al. 1999 [89]								
	- Stratum 1	-0.730	(-1.080, -0.380)	-10.6	(-17.0, -4.3)	-0.610	(-0.960, -0.260)	-13.7	(-25.0, -2.1)
	- Stratum 2	-0.500	(-0.850, -0.150)	-8.1	(-16.0, -0.1)	-0.350	(-0.710, -0.010)	-8.6	(-18.0, -0.8)
	Hallikainen et al. 2000 i [90]	-0.460	(-0.790, -0.130)	-7.3	(-10.4, -4.2)	-0.440	(-0.590, -0.280)	-10.4	(-14.1, -6.7)
	Hallikainen et al. 2000 ii [91]	-0.450	(-0.980, -0.060)	-6.8	(-11.0, -2.7)	-0.270	(-0.800, 0.260)	-5.6	(-11.2, 0.6)

	Hallikainen et al. 2006 [92]	-0.370	(-0.820, 0.080)	-6.0	(-9.6, -2.4)	-0.410	(-0.820, 0.000)	-9.0	(-14.0, -3.6)
	Hallikainen et al. 2008 [93]	-0.590	(-1.500, 0.330)	-10.8	(-22.0, 0.0)	-0.520	(-1.520, 0.480)	-18.6	(-4.6, 9.0)
	Miettinen et al. 1995 [99]								
	- Stratum 1	-0.520	(-0.910, -0.130)	-9.0	(-16.0, -0.9)	-0.440	(-0.620, -0.260)	6.2	(-6.3, 19.0)
	- Stratum 2	-0.670	(-1.060, -0.280)	-11.0	(-20.0, -2.4)	-0.540	(-0.750, -0.360)	-15.0	(-25.0, -5.0)
	Plat et al. 2000 [101]								
	- Stratum 1	-0.390	(-0.880, 0.100)	-8.0	(-17.0, 2.0)	-0.370	(-0.510, -0.220)	-12.6	(-24.0, -0.8)
	- Stratum 2	-0.390	(-0.940, 0.160)	-8.0	(-18.0, 2.0)	-0.340	(-0.510, -0.180)	-11.6	(-23.0, 0.2)
	Turpeinen et al. 2012 [106]	-0.410	(-0.700, -0.116)	-7.0	(-14.0, 0.3)	-0.340	(-0.590, -0.085)	-8.9	(-17.0, -1.1)
SS	Gagliardi et al. 2010 [86]	-0.450	(-1.450, 0.550)	-0.8	(-1.9, 0.3)	-0.050	(-0.910, 0.810)	-14.7	(-26.0, -3.3)
	Hendriks et al. 1999 [111]	-0.310	(-0.410, -0.200)	-5.9	(-11.0, -0.4)	-0.260	(-0.360, -0.150)	-8.5	(-17.0, -0.7)
	Lottenberg et al. 2003 [98]	-0.300	(-0.720, 0.119)	-6.9	(-9.0, -4.7)	-0.300	(-0.410, -0.190)	-6.3	(-8.7, -4.0)
	Nigon et al. 2001 [100]	-0.260	(-0.630, 0.110)	-5.9	(-8.6, -3.2)	-0.240	(-0.550, 0.070)	-24.0	(-55.0, 7.0)
	Ras et al. 2014 [102]	-0.570	(-0.740, -0.400)	-9.0	(-17.0, -1.2)	-0.450	(-0.590, -0.320)	-11.7	(-21.0, -3.0)
	Ras et al. 2015 [103]	-0.260	(-0.460, -0.070)	-4.5	(-12.3, 3.0)	-0.260	(-0.400, -0.120)	-26.0	(-40.0, -12.0)
	Simons 2002 [104]	_	_	-6.7	(-10.0, -4.0)			-8.1	(-12.0, -4.0)
	Theuwissen et al. 2009 [105]	-0.490	(-1.370, 0.390)	-6.7	(-13.0, -0.8)	-0.430	(-1.270, 0.410)	-9.5	(-18.6, -0.4)
	Vissers et al. 2000 [109]	-0.190	(-0.310, -0.070)	NR	NR	-0.200	(-0.300, -0.100)	NR	NR
D	Buyuktuncer et al. 2013 [83]	-0.450	(-0.660, -0.240)	-4.5	(-8.3, -0.8)	-0.260	(-0.412, -0.108)	-6.3	(-9.0, -3.7)
	Charest et al. 2004 [112]	-0.600	(-1.290, 0.086)	NR	NR	-0.500	(-1.050, 0.050)	-13.1	(-31.0, 5.0)
	Clifton et al. 2004 [65]	-0.530	(-0.800, -0.260)	-9.7	(-19.5, 0.1)	-0.530	(-0.810, -0.250)	-12.4	(-22.0, -0.3)
	Hyun et al. 2005 [95]	-0.150	(-0.460, 0.160)	-4.4	(-14.2, 5.0)	-0.240	(-0.430, -0.060)	-7.8	(-20.0, 4.0)
	Jauhiainen et al. 2006 [96]	-0.320	(-0.500, -0.150)	-5.8	(-8.8, -2.8)	-0.360	(-0.530, -0.180)	-10.3	(-14.9, -5.7)

Korpela et al. 2006 [97]								
- Stratum 1	-0.460	(-0.750, -0.170)	-6.9	(-11.0, -3.0)	-0.460	(-0.710, -0.210)	-11.2	(-17.0, -5.0)
- Stratum 2	NR	NR	NR	NR	-0.560	(-0.800, -0.320)	-13.7	(-19.6, -8.0)
- Stratum 3	NR	NR	NR	NR	-0.320	(-0.580, -0.060)	-7.8	(-14.0, -1.9)
Vanstone et al. 2002 [107]	-0.610	(-1.260, 0.040)	-7.8	(-17.6, 2.0)	-0.410	(-0.650, -0.170)	-10.2	(-22.0, 2.0)
Vasquez-Trespalacious et al. 2014 [108]	-0.410	(-0.698, -0.122)	-7.2	(-11.1, -3.3)	-0.320	(-0.560, -0.070)	-10.3	(-14.3, -6.4)

[1] Net change calculated by subtracting the mean change in control group from the mean change in the treatment group. Mean change = blood cholesterol value at the end of intervention – blood cholesterol value at baseline).

NR, not reported. Additionally, not enough information was provided in order to estimate absolute change and / or relative change.

Only the first period of cross-over studies were included in the meta-analysis as current practice [62].

RC, rapeseed/canola; SS, sunflower/soybean; D, dairy

Stratum refers to a single treatment group.

Table 4

Absolute (mmol/L) and relative (%) net change per fat group.

Fat group		TC ES	(95% CI)	LDL-cholesterol ES	(95% CI)
RC	mmol/L	-0.383	(-0.435, -0.330)	-0.384	(-0.431, -0.337)
	%	-6.7	(-7.8, -5.6)	-9.7	(-11.4, -8.1)
SS	mmol/L	-0.322	(-0.433, -0.211)	-0.283	(-0.346, -0.220)
	%	-5.5	(-8.3, -2.7)	-9.5	(-12.7, -6.3)
D	mmol/L	-0.395	(-0.491, -0.299)	-0.357	(-0.428, -0.287)
	%	-6.1	(-7.9, -4.4)	-8.9	(-10.6, -7.3)

Pooled absolute and relative net change in TC and LDL-cholesterol assessed using random effects model.
TC, total cholesterol; ES, estimate; CI, confidence interval; LDL, low-density lipoprotein

9. FIGURES

Figure 1

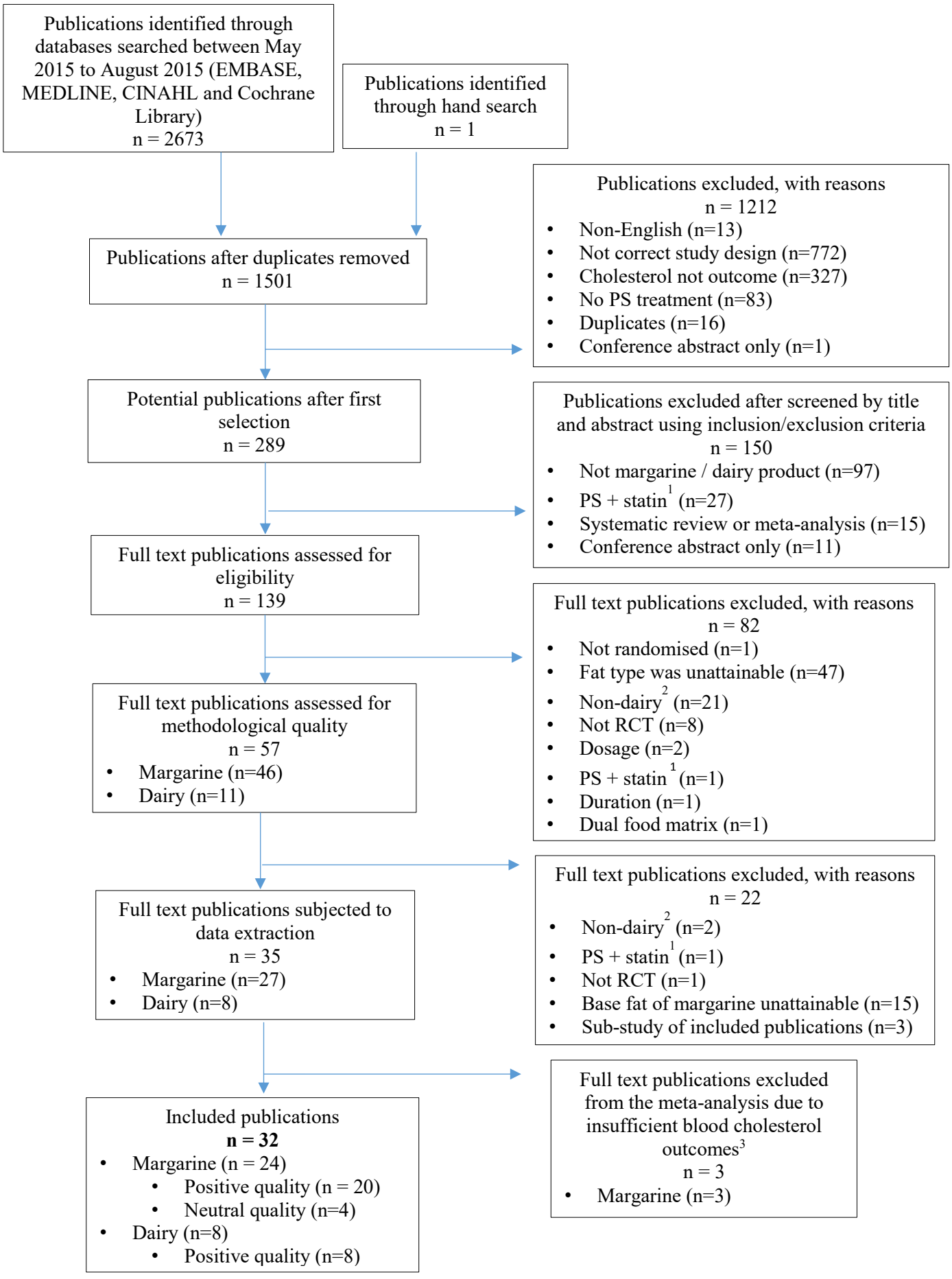


Figure 2

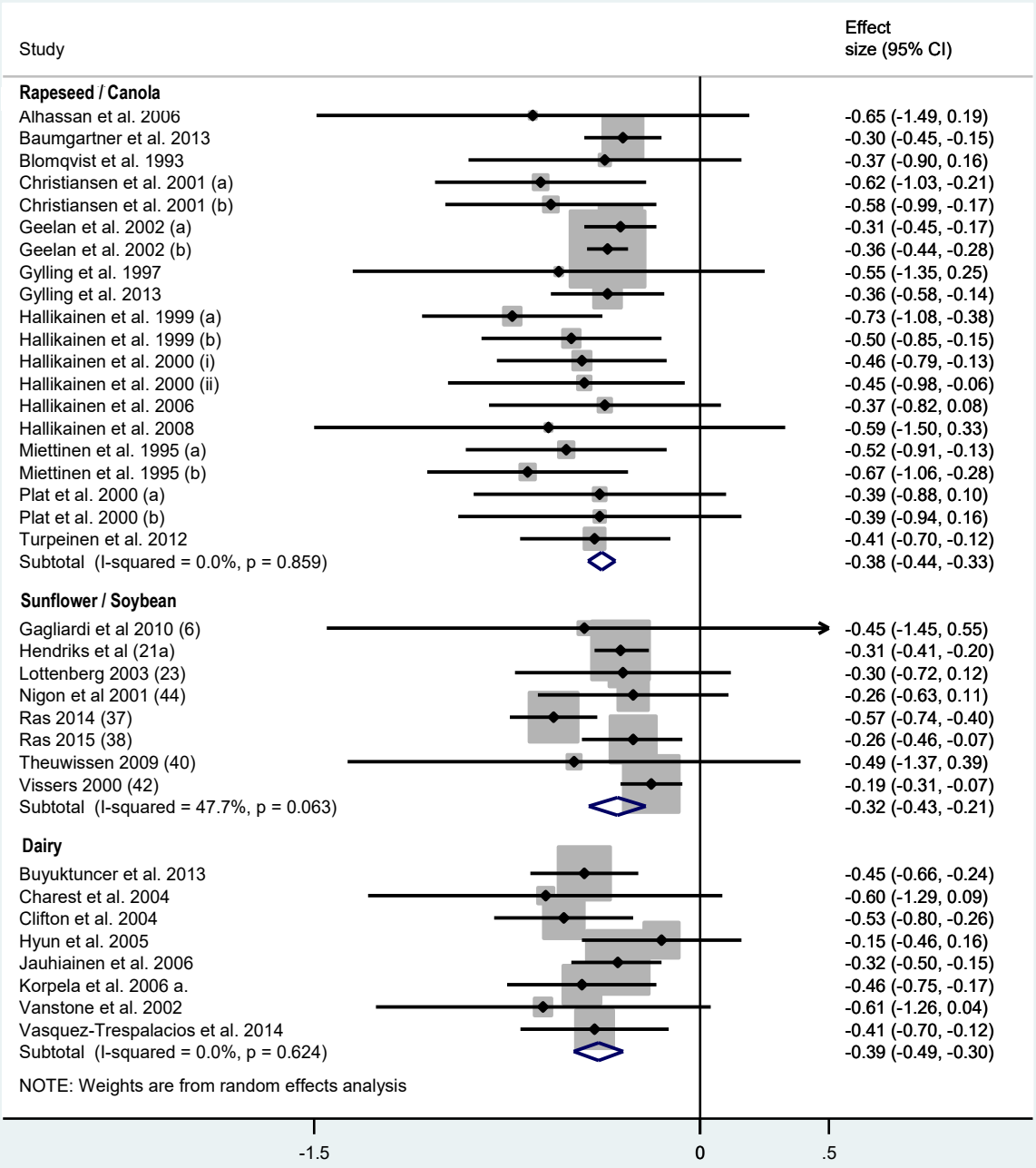
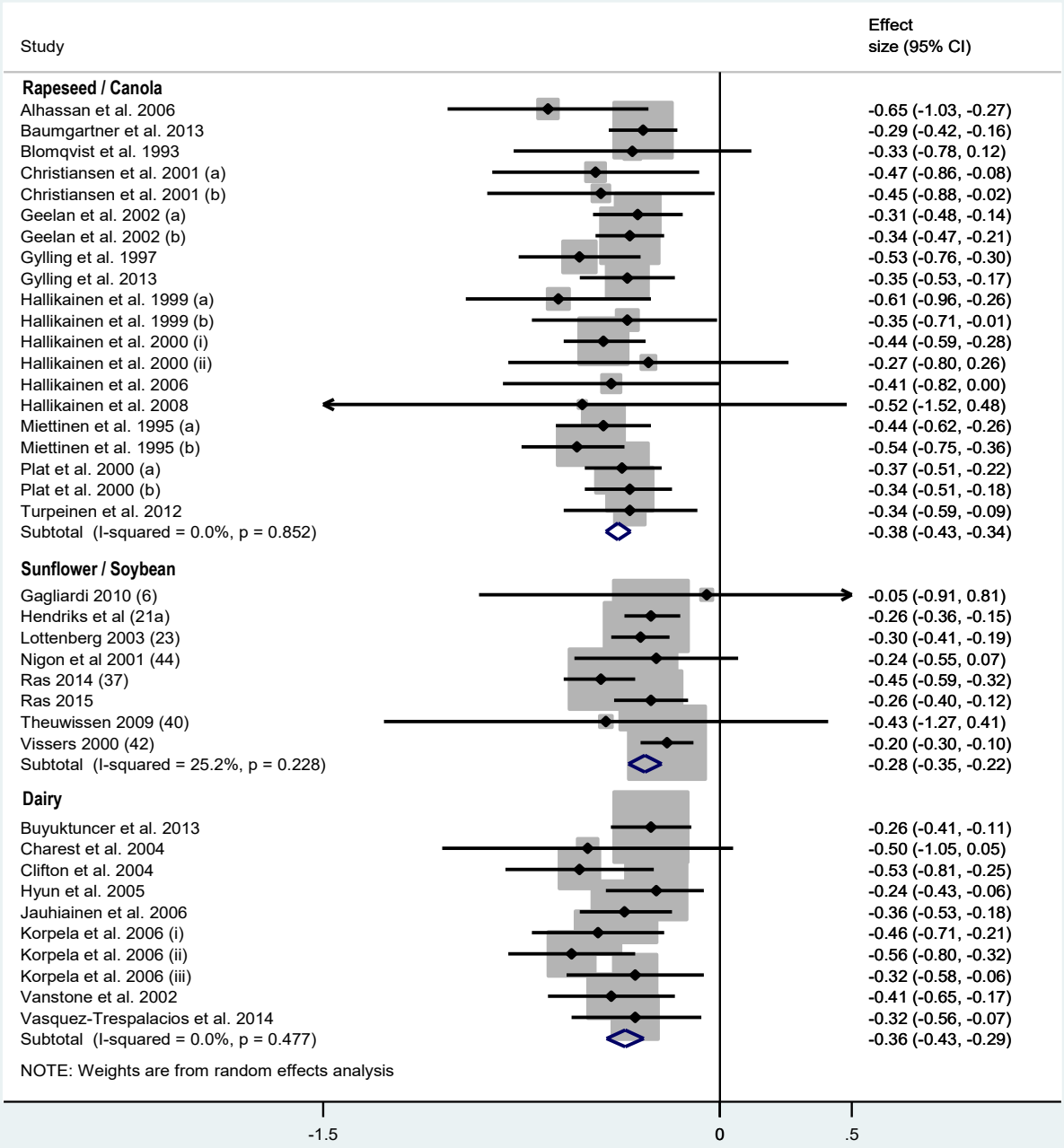

Study
Effect size (95% CI)
Rapeseed / Canola
Alhassan et al. 2006 -0.65 (-1.49, 0.19)
Baumgartner et al. 2013 -0.30 (-0.45, -0.15)
Blomqvist et al. 1993 -0.37 (-0.90, 0.16)
Christiansen et al. 2001 (a) -0.62 (-1.03, -0.21)
Christiansen et al. 2001 (b) -0.58 (-0.99, -0.17)
Geelan et al. 2002 (a) -0.31 (-0.45, -0.17)
Geelan et al. 2002 (b) -0.36 (-0.44, -0.28)
Gylling et al. 1997 -0.55 (-1.35, 0.25)
Gylling et al. 2013 -0.36 (-0.58, -0.14)
Hallikainen et al. 1999 (a) -0.73 (-1.08, -0.38)
Hallikainen et al. 1999 (b) -0.50 (-0.85, -0.15)
Hallikainen et al. 2000 (i) -0.46 (-0.79, -0.13)
Hallikainen et al. 2000 (ii) -0.45 (-0.98, -0.06)
Hallikainen et al. 2006 -0.37 (-0.82, 0.08)
Hallikainen et al. 2008 -0.59 (-1.50, 0.33)
Miettinen et al. 1995 (a) -0.52 (-0.91, -0.13)
Miettinen et al. 1995 (b) -0.67 (-1.06, -0.28)
Plat et al. 2000 (a) -0.39 (-0.88, 0.10)
Plat et al. 2000 (b) -0.39 (-0.94, 0.16)
Turpeinen et al. 2012 -0.41 (-0.70, -0.12)
Subtotal (I-squared = 0.0%, p = 0.859) -0.38 (-0.44, -0.33)
Sunflower / Soybean
Gagliardi et al 2010 (6) -0.45 (-1.45, 0.55)
Hendriks et al (21a) -0.31 (-0.41, -0.20)
Lottenberg 2003 (23) -0.30 (-0.72, 0.12)
Nigon et al 2001 (44) -0.26 (-0.63, 0.11)
Ras 2014 (37) -0.57 (-0.74, -0.40)
Ras 2015 (38) -0.26 (-0.46, -0.07)
Theuwissen 2009 (40) -0.49 (-1.37, 0.39)
Vissers 2000 (42) -0.19 (-0.31, -0.07)
Subtotal (I-squared = 47.7%, p = 0.063) -0.32 (-0.43, -0.21)
Dairy
Buyuktuncer et al. 2013 -0.45 (-0.66, -0.24)
Charest et al. 2004 -0.60 (-1.29, 0.09)
Clifton et al. 2004 -0.53 (-0.80, -0.26)
Hyun et al. 2005 -0.15 (-0.46, 0.16)
Jauhiainen et al. 2006 -0.32 (-0.50, -0.15)
Korpela et al. 2006 a. -0.46 (-0.75, -0.17)
Vanstone et al. 2002 -0.61 (-1.26, 0.04)
Vasquez-Trespalacios et al. 2014 -0.41 (-0.70, -0.12)
Subtotal (I-squared = 0.0%, p = 0.624) -0.39 (-0.49, -0.30)
NOTE: Weights are from random effects analysis
-1.5
0
.5

Figure 3


Study
Effect size (95% CI)
Rapeseed / Canola
Alhassan et al. 2006 -0.65 (-1.03, -0.27)
Baumgartner et al. 2013 -0.29 (-0.42, -0.16)
Blomqvist et al. 1993 -0.33 (-0.78, 0.12)
Christiansen et al. 2001 (a) -0.47 (-0.86, -0.08)
Christiansen et al. 2001 (b) -0.45 (-0.88, -0.02)
Geelan et al. 2002 (a) -0.31 (-0.48, -0.14)
Geelan et al. 2002 (b) -0.34 (-0.47, -0.21)
Gylling et al. 1997 -0.53 (-0.76, -0.30)
Gylling et al. 2013 -0.35 (-0.53, -0.17)
Hallikainen et al. 1999 (a) -0.61 (-0.96, -0.26)
Hallikainen et al. 1999 (b) -0.35 (-0.71, -0.01)
Hallikainen et al. 2000 (i) -0.44 (-0.59, -0.28)
Hallikainen et al. 2000 (ii) -0.27 (-0.80, 0.26)
Hallikainen et al. 2006 -0.41 (-0.82, 0.00)
Hallikainen et al. 2008 -0.52 (-1.52, 0.48)
Miettinen et al. 1995 (a) -0.44 (-0.62, -0.26)
Miettinen et al. 1995 (b) -0.54 (-0.75, -0.36)
Plat et al. 2000 (a) -0.37 (-0.51, -0.22)
Plat et al. 2000 (b) -0.34 (-0.51, -0.18)
Turpeinen et al. 2012 -0.34 (-0.59, -0.09)
Subtotal (I-squared = 0.0%, p = 0.852) -0.38 (-0.43, -0.34)
Sunflower / Soybean
Gagliardi 2010 (6) -0.05 (-0.91, 0.81)
Hendriks et al (21a) -0.26 (-0.36, -0.15)
Lottenberg 2003 (23) -0.30 (-0.41, -0.19)
Nigon et al 2001 (44) -0.24 (-0.55, 0.07)
Ras 2014 (37) -0.45 (-0.59, -0.32)
Ras 2015 -0.26 (-0.40, -0.12)
Theuwissen 2009 (40) -0.43 (-1.27, 0.41)
Vissers 2000 (42) -0.20 (-0.30, -0.10)
Subtotal (I-squared = 25.2%, p = 0.228) -0.28 (-0.35, -0.22)
Dairy
Buyuktuncer et al. 2013 -0.26 (-0.41, -0.11)
Charest et al. 2004 -0.50 (-1.05, 0.05)
Clifton et al. 2004 -0.53 (-0.81, -0.25)
Hyun et al. 2005 -0.24 (-0.43, -0.06)
Jauhiainen et al. 2006 -0.36 (-0.53, -0.18)
Korpela et al. 2006 (i) -0.46 (-0.71, -0.21)
Korpela et al. 2006 (ii) -0.56 (-0.80, -0.32)
Korpela et al. 2006 (iii) -0.32 (-0.58, -0.06)
Vanstone et al. 2002 -0.41 (-0.65, -0.17)
Vasquez-Trespalacios et al. 2014 -0.32 (-0.56, -0.07)
Subtotal (I-squared = 0.0%, p = 0.477) -0.36 (-0.43, -0.29)
NOTE: Weights are from random effects analysis
-1.5
0
.5

10. FIGURE LEGENDS

Figure 1

Flow diagram of publication selection process starting with 2673 publications and ending with 24 RCT.

[1] PS plus cholesterol-lowering therapy without PS-placebo (i.e. PS alone) treatment arm

[2] Fat type of 'dairy' product was non-representative of commercial animal fat-based dairy products

[3] These include publications which insufficient data available which were not able to be provided by the authors.

Figure 2

Effect size and 95% CI for TC concentrations according to fat type in PS fortified food.

Figure 3

Effect size and 95% CI for LDL-C concentrations according to fat type in PS fortified food.