



FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Systematic Review of the Evidence for a Relationship between Phytosterols and Blood Cholesterol

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Executive Summary

<i>Does intake of phytosterols, phytostanols and their esters affect blood cholesterol?</i>	
Food-health relationship	Phytosterol intake reduces blood total and LDL cholesterol concentrations
Degree of certainty (GRADE rating)	⊕⊕⊕⊕ High
Component	Notes
Body of evidence	125 randomised controlled trials (RCTs) involving 9128 participants were considered; 107 of these trials were combined in two existing meta-analyses. LDL cholesterol data were reviewed from 125 RCTs, and total cholesterol data were reviewed from 60 RCTs.
Consistency	Reduced total and LDL cholesterol concentrations following increased phytosterol intake was almost universally reported, with the majority of trials finding statistically significant decreases over a range of doses. The effect was present in people with normal cholesterol concentrations.
Causality	The RCTs considered were placebo-controlled and this study design provides a high degree of certainty for a causal relationship.
Plausibility	Phytosterols are structurally similar to cholesterol and compete for intestinal absorption. This limits the uptake of cholesterol in the gut and so leads to reduced blood cholesterol concentrations.
Generalisability	The RCTs have been conducted in America, Europe, Australia and Asia, making the results applicable to the Australian and New Zealand populations.

The purpose of this review was to assess the currency of the pre-approved high level health claim that phytosterols, phytostanols and their esters (collectively referred to as phytosterols) ‘reduce blood cholesterol’. In performing this check for currency, FSANZ has followed the requirements for updates to existing systematic reviews, as set out in the *Application Handbook* and in Schedule 6 of Standard 1.2.7 – Nutrition, Health and Related Claims.

Two systematic reviews, each with a meta-analysis, were selected as the starting point for assessing the food-health relationship. Together, these reviews pooled data from 107 randomised controlled trials (RCTs). The meta-analyses estimated that intake of 1.6 g to 2.2 g phytosterols per day resulted in an approximately 0.33 mmol/L decrease in LDL cholesterol concentration and a 0.36mmol/L decrease in total cholesterol concentration. FSANZ identified 19 trials published since the reviews, all of which lay within the band of dose-response of the existing studies and were broadly consistent with the effect estimates from the meta-analyses.

Overall, the body of evidence was considered to be of high quality, with minimal risk of bias. Using the GRADE framework, it was concluded that there is a ‘High’ degree of certainty that increased phytosterol intake reduces blood total and LDL cholesterol concentrations, and that the pre-approved high level health claim remains current.

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1 Introduction

The currency of pre-approved high level health claims is being considered during the transition period for Standard 1.2.7 – Nutrition, Health and Related Claims. The relationship between phytosterols, phytostanols and their esters with blood cholesterol concentrations has previously been considered by FSANZ in applications to add these compounds as novel foods to the food supply. Based on the assessment of these applications, and similar international health claims, the relationship was included as a pre-approved high level health claim in Standard 1.2.7 (See Table 1).

Table 1 *Pre-approved high level health claim for phytosterols in Schedule 2 of Standard 1.2.7*

Food or property of food	Specific health effect	Relevant population	Context claim statements	Conditions
Phytosterols, phytostanols and their esters	Reduces blood cholesterol		Diet low in saturated fatty acids Diet containing 2 g of phytosterols, phytostanols and their esters per day	The food must – (a) meet the relevant conditions specified in Columns 1 and 2 of the Table to clause 2 in Standard 1.5.1; and (b) contain a minimum of 0.8 g total plant sterol equivalents content per serving

In this review the evidence for the relationship between phytosterols, phytostanols and their esters with blood cholesterol concentrations has been assessed by updating two recent systematic reviews.

1.1 Property of food

Phytosterols, or plant sterols, are structurally similar to cholesterol with the addition of a side chain at C24. Phytostanols, or plant stanols, are the saturated form of phytosterols. Both these molecules can be esterified to a fatty acid at the hydroxyl group to form phytosterol esters or phytostanol esters. For simplicity, “phytosterols” is used to refer to phytosterols, phytostanols and their esters throughout this document, except when noted.

Phytosterols, as their name implies, are found in plant foods, with the most common being sitosterol and campesterol. Vegetable oils can be rich sources of phytosterols. In addition, phytosterols can be extracted from tall oils which are derived from pine trees. In Australia and New Zealand, it is permitted to add phytosterols, phytostanols and their esters to edible oil spreads, breakfast cereals, milk and yoghurt. The permitted amounts supply an amount of phytosterol equivalents ranging from 0.75 to 1 g per serving.

1.2 Health effect

In Schedule 2 of Standard 1.2.7 the health effect is ‘reduces blood cholesterol’. Reductions in total and low density lipoprotein (LDL) cholesterol are considered to be a beneficial health effect due to elevated levels of these blood lipids being risk factors for coronary heart disease (CHD). In contrast, although high density lipoprotein (HDL) cholesterol concentrations are inversely related to CHD, their predictive power for CHD incidence is less certain.

Total cholesterol can be measured in serum or plasma. Following saponification to release free cholesterol from cholesterol esters, cholesterol is then extracted and measured using a colourimetric reaction. LDL cholesterol can be measured directly following separation by ultracentrifugation, or, more commonly, is calculated from direct measures of total, HDL and triglyceride levels using the Friedewald equation¹.

Hypercholesterolaemia is described in Australia as being total serum cholesterol concentrations ≥ 5.5 mmol/L.² The normal range for LDL cholesterol is described as 2.0-3.4 mmol/L by some³ and < 3.5 mmol/L by others⁴. Because studies often report averages to two decimal places, this report uses < 3.5 mmol/L as the definition of normal LDL cholesterol concentration.

1.3 Proposed relationship

The proposed food-health relationship is that increased consumption of phytosterols, phytosterols and or their esters reduces blood cholesterol concentrations. Specifically, it is the reduction of total and or LDL cholesterol that represent a beneficial health effect, whereas reductions in HDL cholesterol are considered an adverse health effect. In the dietary context for the pre-approved high level health claim, consumption of 2 g phytosterols per day is recommended to give the health effect.

2 Summary and critical appraisal of an existing systematic review

Searching for systematic reviews on the relationship between phytosterol intake and blood cholesterol identified multiple reviews (Abumweis et al. 2008; Demonty et al. 2009; Wu et al. 2009; Musa-Veloso et al. 2011; Genser et al. 2012; Cusack et al. 2013; Ras et al. 2013; Shaghghi et al. 2013). The reviews varied in their purpose, literature search strategies and eligibility criteria. Two reviews written by authors from the same organisation were selected for updating (Demonty et al. 2009; Ras et al. 2013). The inclusion of quality assessment of trials included in the first review was a key consideration in its use. Both reviews were from authors employed by Unilever, which makes a variety of phytosterol-enriched products. However, there was a high degree of consistency in the conclusions and effect estimates between different reviews, albeit most reviews had industry-affiliated authors.

The first review assessed the dose-response relationship between phytosterol intake and LDL cholesterol concentrations. This review was published in 2009, with the literature search performed in 2007 (Demonty et al. 2009). The more recent review, published in 2013, assessed the effects of phytosterol intake on blood phytosterol levels, with effects on total and LDL cholesterol concentrations also reported (Ras et al. 2013). The second review used a similar search strategy, with the electronic searches performed in June 2012. The eligibility criteria were also very similar between reviews. However, some potentially relevant articles were excluded from the second review as they did not report blood phytosterol levels. The list of articles excluded on this basis was provided to FSANZ by the review authors and were included in this update to the review.

¹Friedewald equation calculates LDL cholesterol using the following formula:

LDL = total cholesterol – HDL cholesterol – (triglyceride / 2.2) where all concentration are in mmol/L

² This cut-off point is used in the Australian Health Survey and by the Therapeutic Goods Administration <https://www.tga.gov.au/book/part-b-further-technical-guidance> accessed 22 September 2015

³http://www.rcpamanual.edu.au/index.php?option=com_pttests&task=show_test&id=450&Itemid=34 accessed 21 October, 2014

⁴http://www.swsld.nsw.gov.au/sswps/handbook/Results4.asp?Test_ID=3094&Org_ID=&Query_TEXT=&TEST_GRP=LIPID+TESTS&DISEASE= empty12&ORGLAB= empty12&R1 accessed 21 October, 2014.

In the critical appraisal the reviews were discussed together where appropriate, with differences in the reviews noted.

2.1 Methods used in the existing review

2.1.1 Study selection

Both reviews used search terms for phytosterol and phytostanols and restricted the searches to human studies and clinical trials where possible. The search terms for outcomes varied, with the review by Demonty et al. (2009) searching for cholesterol outcomes, while Ras et al. (2013) searched for blood outcomes by using the terms “blood*”, “plasma” and “serum” (see Appendix 1). Ras et al. (2013) used these search terms as they were interested in blood phytosterol levels, which are often reported as secondary outcomes in trials. These search terms would also have captured reports on blood cholesterol outcomes which are of interest to this review.

Electronic searches were performed by Demonty et al. (2009) in the following databases:

- MEDLINE
- Cab Abstracts
- Biological abstracts
- Web of Science
- Cochrane Library.

Ras et al. (2013) searched in the first two databases listed above, as well as:

- EMBASE
- Food Science & Technology abstracts
- HCA Plus
- Biosis.

2.1.2 Eligibility criteria

The eligibility criteria are summarised in Table 2. Concomitant interventions with statins, low-fat diets, vegetable oil-rich background diet or phytosterols esterified to vegetable oil fatty acids were included if the concomitant intervention was the same in the experimental and control groups.

Table 2 *PICOT criteria for study selection used by Demonty et al. (2009) and Ras et al. (2013)*

Population	Human adults (age not specified)
Intervention	Randomised controlled trial using phytosterols, phytostanols or their esters
Comparator	Placebo required in control arm (Ras review)
Outcomes	Demonty review: blood lipids (primary outcome was LDL cholesterol) Ras review: blood phytosterols (sitosterol and campesterol), with cholesterol as secondary outcomes
Time	≥2 weeks

Trials were excluded if the phytosterol dose was greater than 10 g per day, or if ferulated phytosterols were used (phytosterols conjugated to ferulic acid, found in rice bran oil or shea nut oil). Trials in colectomised patients were also excluded. In the Ras et al. (2013) review, trials were excluded if the intervention included >20% of phytosterol mix being phytostanols, or did not report serum phytosterol levels. These exclusion criteria are not relevant to the current assessment of the food-health relationship. Therefore, the articles excluded by Ras

et al. (2013) under these criteria were considered in Section 3 which provides an update of the reviews.

2.1.3 Quality assessment

The quality of included studies was appraised in the Demonty et al. (2009) review using a customised tool to give a numerical score. The tool considered 'random sequence generation, blinding of the subjects, blinding of the investigators, eligibility criteria specified, compliance, and carryover effects taken care of in case of cross-over trials'. Based on the quality score, studies were stratified as 'good' or 'low' quality.

Ras et al. (2013) did not assess quality of individual studies with the authors stating that exclusion based on a subjective quality analysis was inappropriate.

2.2 Summary of results

The review and meta-analysis by Demonty et al. (2009) included 84 trials, with 141 strata and 6805 participants. The primary outcome was LDL cholesterol concentrations. All except two strata showed a reduction in LDL cholesterol concentrations with phytosterol intake, with these reductions significant in 109 strata. Meta-analysis demonstrated that a mean daily intake of 2.15 g free phytosterol equivalent intake reduced LDL-C concentrations by 0.34 mmol/L (95% CI: -0.36, -0.31), with the relative difference -8.8% (95% CI: -9.4%, -8.3%). Effect estimates were not calculated for total cholesterol.

Demonty et al. (2009) also calculated dose response curves and the effects of various study parameters on these curves were estimated. The dose response curve predicted that a daily intake of 2 g phytosterol per day would reduce LDL cholesterol by 0.35 mmol/L, or 9%, both of which are consistent with the effect estimates of the meta-analysis. Effects plateaued at doses above 3 g per day. Comparison of covariates found no effect of the following on the absolute dose-response curves (in mmol/L):

- Type of phytosterol (plant sterol vs. plant stanol)
- Food format (non-fat vs. fat based, dairy vs. non-dairy, solid vs. liquid)
- Study quality (high vs. low quality, high vs. low compliance, well vs. poorly randomised)
- Study design (parallel vs. cross-over).

In the review by Ras et al. (2013), only those studies that reported blood phytosterol levels were included. Therefore, fewer studies (41 trials, 55 strata) were included in this meta-analysis. Of these, 18 trials reporting 24 strata with 1169 participants were common to the review by Demonty et al. (2009). The Ras et al. (2013) meta-analysis reported that a mean daily intake of 1.6 g free phytosterol equivalents reduced LDL cholesterol by 0.33 mmol/L (95% CI: -0.37, -0.30) and total cholesterol by 0.36 mmol/L (95% CI: -0.40, -0.32). Intake of phytosterols did not affect HDL cholesterol concentrations (-0.00 mmol/L [95% CI: -0.02, 0.01]). Subgroup analysis demonstrated a significant effect of baseline LDL cholesterol concentrations and phytosterol dose, with greater reductions observed with higher baseline cholesterol concentrations (see Table 3).

Table 3 Summary of total and LDL cholesterol findings from Ras et al. (2013) meta-analysis

Subgroup		Total Cholesterol			LDL Cholesterol		
		Effect estimate	95% CI	p-value for subgroup difference	Effect estimate	95% CI	p-value for subgroup difference
All trials		-0.36	-0.40, -0.32	n.a.	-0.33	-0.37, -0.30	n.a.
Baseline cholesterol ¹	Below median	-0.26	-0.32, -0.20	<0.001	-0.26	-0.31, -0.21	0.001
	Above median	-0.41	-0.45, -0.37		-0.37	-0.41, -0.34	
Phytosterol dose (g per day)	≥0.3 and ≤1.5	-0.28	-0.36, -0.20	0.039	-0.25	-0.32, -0.18	0.038
	>1.5 and <2.0	-0.35	-0.41, -0.30		-0.35	-0.40, -0.30	
	≥2.0 and ≤3.2	-0.40	-0.46, -0.35		-0.35	-0.40, -0.31	

¹Median baseline total cholesterol concentration was 6.0 mmol/L, and LDL cholesterol was 3.9 mmol/L

2.3 Critical appraisal of the existing review

Overall, both reviews are of a high quality and are suitable for use as a starting point for evaluating the relationship between phytosterol intake and blood cholesterol concentrations. The Ras et al. (2013) review may have excluded relevant trials based on their requirement for blood phytosterol levels as an outcome. However, this limitation was overcome by the authors providing the list of studies excluded on this criterion to FSANZ.

2.3.1 Study identification and selection

Both reviews used a broad search strategy which enabled relevant literature to be captured. Sources of grey literature were not searched for additional material. The eligibility criteria were also appropriate with relevant phytosterol doses and duration of intervention considered. Because the 2-week minimum duration inclusion criteria may underestimate some effects, the minimum duration of included trials was 2 weeks. The Ras et al. (2013) review had some limitations in the eligibility criteria with respect to the current purpose of assessing the food health relationship, in that only studies that reported blood phytosterol levels were included, and trials with the interventions including >20% of the phytosterol mix being phytosterols, were excluded. However, the studies excluded on these criteria were provided to FSANZ. Therefore it is likely that the use of both reviews captured all relevant literature published up until June 2012.

2.3.2 Assessment of bias

Quality assessment was performed by Demonty et al. (2009) using a customised tool to give a numerical score. The score (described above) included assessment of selection and performance bias, but reporting and attrition bias were not specifically addressed. The funnel plot indicated an absence of publication bias for blood cholesterol outcomes in both reviews.

The quality assessment score was used to classify studies as good or low quality based on a score threshold. In addition to comparing outcomes from good and low quality studies, results were compared between studies with adequate and inadequate randomisation and high and low compliance. For all of these comparisons, no significant difference was found for the absolute or relative changes in either LDL cholesterol or the dose step required to achieve an additional effect.

The Ras et al. (2013) review did not assess individual study quality. Given the quality analysis in the larger Demonty et al. (2009) review found no significant effect of study quality, its absence was not considered to be a major limitation of the review by Ras et al. (2013).

2.3.3 Data extraction and analysis

For both reviews, authors extracted baseline and end of intervention data for blood cholesterol concentrations (total, LDL and HDL cholesterol). Change data were calculated using appropriate formulae which were described in supplementary data for both reviews. The supplement⁵ for the review by Demonty et al. (2009) giving the formulae included some typographical errors, but these were corrected in the subsequent review by Ras et al. (2013).

Weighted, random effects meta-analyses were used to calculate pooled effect estimates for both absolute (mmol/L) and relative (%) changes in both reviews. Weighting was done using the inverse variance of the study results. Demonty et al. (2009) also calculated a dose-response curve, which produced results consistent with the effect estimate. The analysis methods in both reviews were appropriate.

Demonty et al. (2009) also assessed the effect of categorical covariates, specifically the type of phytosterol, food format, study quality and design on the dose-response curve. No significant effects of these variables were found for absolute change. Solid compared to liquid food formats were found to have a greater effect on LDL cholesterol reduction in the relative dose-response curve. The data also suggested a greater effect with multiple daily dosing, however this was partly confounded by differences in the total daily dose between the studies in the review.

2.3.4 Data interpretation

The reviews by Demonty et al. (2009) and Ras et al. (2013) concluded that an average intake of 1.6 g or 2.2 g free phytosterol equivalents per day reduced blood LDL cholesterol concentrations by 0.34 or 0.33 mmol/L, respectively. These conclusions were well supported by the data. The narrow confidence intervals and highly significant *p*-values demonstrate the high degree of certainty in the pooled effect estimates. The overall quality of the evidence base was not rated, but the consistency of effect is clear. Similar comments apply to the reduction in total cholesterol concentration of 0.36mmol/L reported by Ras et al. (2013).

2.4 Consideration of validity and strength of evidence

The reviews by Demonty et al. (2009) and Ras et al. (2013) used an overlapping evidence base to draw the same conclusion. These reviews provide a high degree of certainty in the relationship between phytosterol intake and reduced LDL-C concentrations. Furthermore, other recent systematic reviews have drawn similar conclusions despite different review objectives, such as a comparative analysis of plant sterols and stanols (Musa-Veloso et al. 2011), or assessment of cholesterol-lowering efficacy of phytosterols in a capsule or tablet format (Shaghghi et al. 2013). In totality, the evidence base of studies published prior to June 2012 supports the substantiation of the food-health relationships for both total and LDL cholesterol concentrations with a high degree of certainty.

3 Evaluation of new evidence

The search strategies used in the studies by Demonty et al. (2009) and Ras et al. (2013) were very similar, with the search by Ras et al. (2013) performed in June 2012. In this section, the literature is searched for studies published after this date. Studies meeting the eligibility criteria were then assessed for quality, and the impact of their outcomes on the food-health relationship considered.

⁵ The supplement is available at <http://jn.nutrition.org/content/suppl/2009/01/19/jn.108.095125.DC1/nut095125SAPP02.pdf>

3.1 Methods

3.1.1 Search strategy

The search strategy from the Demonty et al. (2009) review was selected as it included terms for cholesterol outcomes. Searches were performed in EMBASE, PubMed, Food Science and Technology Abstracts and Cochrane CENTRAL (see Appendix 1). Based on the Ras search date, searches were restricted to 2012 to present and June 2012 to present in the PubMed database (see Appendix 1 for details).

To ensure all relevant publications were captured, the articles excluded by Ras et al. (2013) for not having blood phytosterol outcomes, or for interventions with >20% of phytosterol mix being phytosteranols, were requested from Unilever. Of the 83 publications provided, 33 were published since the searches performed in the review by Demonty et al. (2009). These 33 publications were screened for inclusion.

3.1.2 Inclusion and exclusion criteria

The eligibility criteria used by Demonty et al. (2009) were used, with minor modification (see Table 4). In addition, studies were excluded that administered phytosterols in capsule form, or involved a concomitant intervention involving a drug or dietary supplement in the form of a capsule. These criteria were added to restrict the consideration of new evidence to that which was relevant to the assessment of the food-health relationship.

Table 4 PICOTS criteria used in FSANZ update of Demonty et al. (2009) and Ras et al. (2013) reviews

Population	Human adults (≥ 18)
Intervention	Phytosterols, phytosteranols or their esters in a food-based format
Comparator	Placebo required in control arm
Outcomes	Total, LDL and or HDL cholesterol
Time	≥ 3 weeks
Study type	Randomised controlled trial

3.1.3 Unpublished material

The reference lists of full-text publications were screened for reports of relevant trials.

3.1.4 Study selection

Records identified by the search strategy were imported into EPPI-Reviewer 4 (<http://eppi.ioe.ac.uk/cms/er4>). Following removal of duplicates, records were screened on title and abstract. Candidate full-text articles were retrieved and assessed compared to the selection criteria. Data were extracted using a standard form. Screening and data extraction were conducted by one investigator, with data extraction verified by a second investigator.

3.1.5 Data extraction and statistical analyses

A tiered approach was used to determine the level of data extraction required to assess the impact of new evidence on the conclusions of the Ras et al. (2013) and Demonty et al. (2009) reviews. The change in LDL cholesterol was extracted into a table to enable comparison of outcomes with published meta-analysis results. The purpose of this update was to examine the currency of the relationship between increased phytosterol consumption and blood cholesterol. Given the new data are consistent with the meta-analysis effect estimates, and clearly demonstrate that increasing phytosterol consumption reduces blood

total and LDL cholesterol concentrations, recalculation of the overall effect size was not considered necessary.

Demonty et al. (2009) did not include a sub-analysis by baseline cholesterol status of the participants. Ras et al. (2013) divided the studies using the median value from all studies (6.0mmol/L). Therefore this does not specifically address the question of whether the effect is seen in people with normal cholesterol concentrations. In Australia, normal LDL cholesterol is sometimes described as 2.0-3.4mmol/L⁶ and sometimes as <3.5mmol/L⁷. Because some studies report data to two decimal places, the current report defines studies in which the mean baseline LDL cholesterol was <3.5mmol/L as having been done in normocholesterolaemic subjects, regardless of how study authors described their populations. The value used was the overall baseline for the total study population, when available or a weighted average baseline value for studies in which data are given separately for intervention and control groups. Baseline values were used in preference to screening values where both types were given. In addition the HET group from Myrie et al. (2012) was excluded due to selection for possible phytosterolaemia. A further restriction was that studies in which all subjects were using cholesterol-lowering medication such as statins when the baseline specimen was taken were excluded from the definition of normocholesterolaemia and therefore from the analysis.

The mean difference in LDL cholesterol and its 95% CI were extracted from the table in Demonty et al. (2009) for studies conducted in normocholesterolaemic subjects except for Lau et al. (2005) where there was a mismatch between the mean and its 95% CI. Data were extracted from the original papers for studies published since the Demonty et al. (2009) review. Results from an ANCOVA analysis were preferentially extracted where these were available. Following data extraction, changes in blood cholesterol concentration were calculated if change values were not reported. For cross-over studies the change in blood cholesterol concentration were calculated as:

$$\text{Change} = \text{Cholesterol}_{(\text{end of intervention})} - \text{Cholesterol}_{(\text{end of control})}$$

The error for the change in cholesterol concentration in cross-over trials was calculated as⁸:

$$\text{SE} = \sqrt{[(\text{SE}_{(\text{end of intervention})})^2 + \text{SE}_{(\text{end of control})}^2) - 2r(\text{SE}_{(\text{end of intervention})})(\text{SE}_{(\text{end of control})})]}$$

For parallel studies, the change in blood cholesterol was calculated as:

$$\text{Change} = (\text{Cholesterol}_{(\text{end, intervention})} - \text{Cholesterol}_{(\text{baseline, intervention})}) - (\text{Cholesterol}_{(\text{end, control})} - \text{Cholesterol}_{(\text{baseline, control})})$$

The error for the change in cholesterol concentration in parallel trials was calculated as:

$$\text{SE} = \sqrt{(\text{SE}_1 + \text{SE}_2)}, \text{ where}$$

$$\text{SE}_1 = \sqrt{[(\text{SE}_{(\text{end, intervention})})^2 + \text{SE}_{(\text{baseline, intervention})}^2) - 2r(\text{SE}_{(\text{end, intervention})} \times \text{SE}_{(\text{baseline, intervention})})]}$$

$$\text{SE}_2 = \sqrt{[(\text{SE}_{(\text{end, control})})^2 + \text{SE}_{(\text{baseline, control})}^2) - 2r(\text{SE}_{(\text{end, control})} \times \text{SE}_{(\text{baseline, control})})]}$$

⁶ http://www.rcpamanual.edu.au/index.php?option=com_pttests&task=show_test&id=450&Itemid=34 accessed 21 October, 2014

⁷ http://www.swslhd.nsw.gov.au/sswps/handbook/Results4.asp?Test_ID=3094&Org_ID=&Query_TEXT=&TEST_GRP=LIPID+TESTS&DISEASE= empty12&ORGLAB= empty12&R1= accessed 21 October, 2014)

⁸ SE, standard error; r=0.8 was used as the correlation coefficient for measures of blood cholesterol (Demonty et al. 2009) .

Cholesterol data reported in mg/dL were converted to mmol/L by dividing by 38.6. $r=0.8$ was used as the correlation between repeated measures of cholesterol (Demonty et al. 2009). Studies which reported results only as % change were excluded from the meta-analysis.

Meta-analyses were performed using a random effects model and generic inverse variance method to allow combination of the varied data reporting methods, and to ensure cross-over studies were not given less weight compared to parallel studies. Data were analysed using Review Manager version 5.3, the systematic review software developed by The Cochrane Collaboration (The Nordic Cochrane Centre 2014).

3.2 Results

3.2.1 Search results

The identification of studies is summarised in Figure 1. Screening the reference lists of included publications did not identify any additional trials. The large number of trials from “other sources” was the publications provided by Unilever which were excluded from the review by Ras et al. (2013) as they did not report blood phytosterol levels. Searching the ICTRP identified 77 completed and ongoing trials since 2005. More recently, nine trials were registered in 2011, 13 in 2012, seven in 2013 and two in 2014. Results of three of the trials registered since 2011 were reported in the publications included in the FSANZ update.

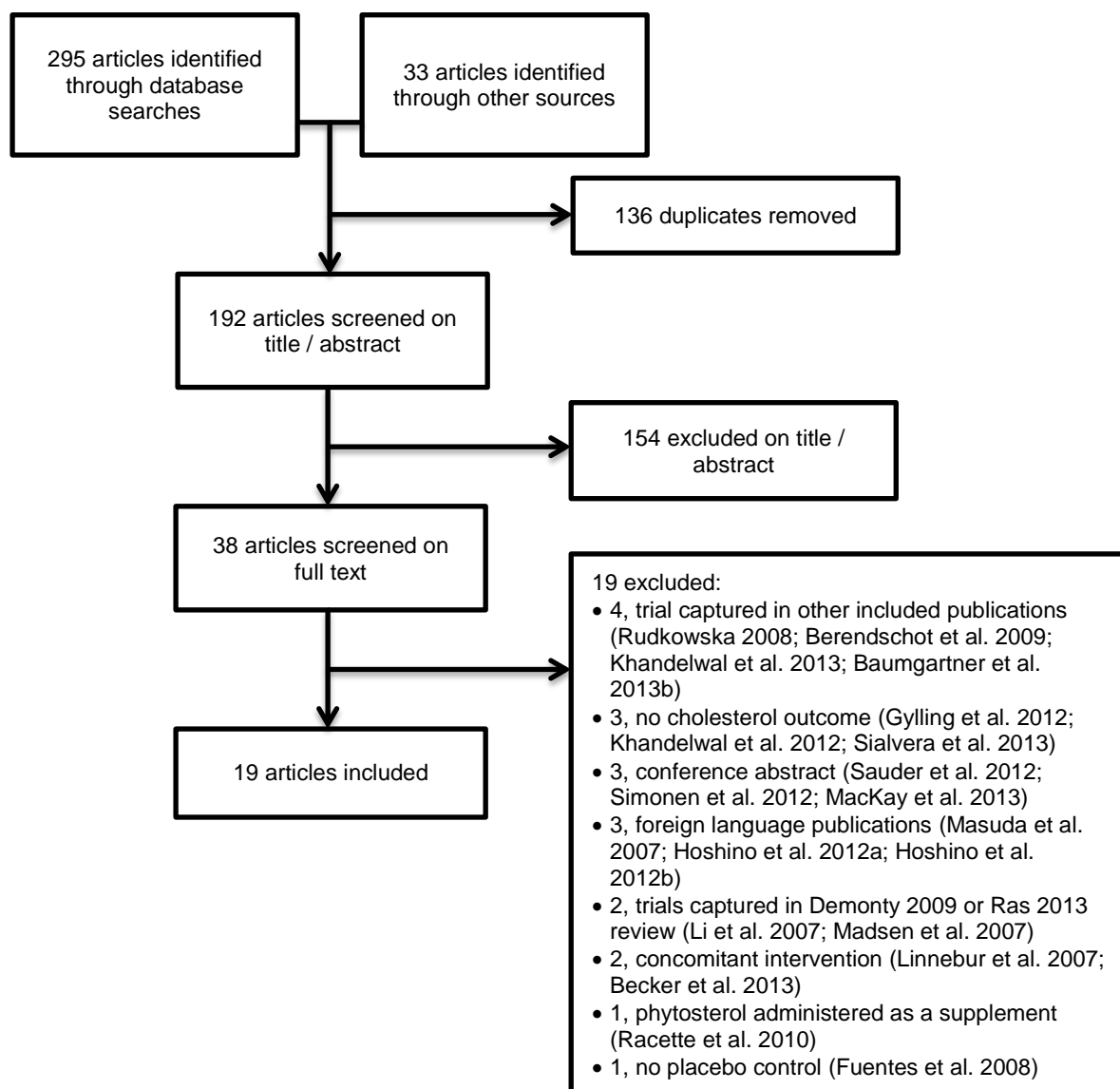


Figure 1 PRISMA diagram for trials included in the review update

3.2.2 Included studies

Nineteen trials were included in the update of the systematic reviews by Demonty et al. (2009) and Ras et al. (2013). Individual trial details are summarised in Appendix 2. Fourteen trials used a parallel design (Goncalves et al. 2007; Weidner et al. 2008; Gylling et al. 2009; Khandelwal et al. 2009; Banuls et al. 2010; Gagliardi et al. 2010; Banuls et al. 2011; Dulalia et al. 2011; Hernandez-Mijares et al. 2011; Sialvera et al. 2012; Soderholm et al. 2012; Buyuktuncer et al. 2013; Gylling et al. 2013; Hallikainen et al. 2013), and there were five cross-over trials (Allen et al. 2008; Ruiu et al. 2009; Chen et al. 2009; Baumgartner et al. 2013a; Shaghghi et al. 2014). The majority of trials were in populations with hypercholesterolaemia, or with the majority of participants having hypercholesterolemia. Two trials were in subjects with normal blood lipids (Soderholm et al. 2012; Baumgartner et al. 2013a), and three selected participants with metabolic syndrome (Gagliardi et al. 2010; Hernandez-Mijares et al. 2011; Sialvera et al. 2012). One trial was conducted in subjects with familial hypercholesterolemia (Ruiu et al. 2009), a genetic condition causing severe elevations in blood cholesterol concentrations.

Trial duration is summarised in Table 5, with the most common duration being 4 weeks. Fourteen trials tested the effects of plant sterols, three tested plant stanols, and two tested both. The doses ranged from 1.6 to 4.0 g plant sterol or stanol per day (see Table 5). Seven studies were industry funded, four were government funded, four received mixed industry and government funding. The funding source was unclear for the remaining four trials. No serious adverse effects were reported with phytosterol consumption.

Figure 2 shows the effect sizes of the newly identified studies overlaid on Figure 3A (showing the phytosterol dose/change in LDL cholesterol) from Demonty et al. (2009) for those studies giving data as absolute data (mmol/L) rather than relative differences.

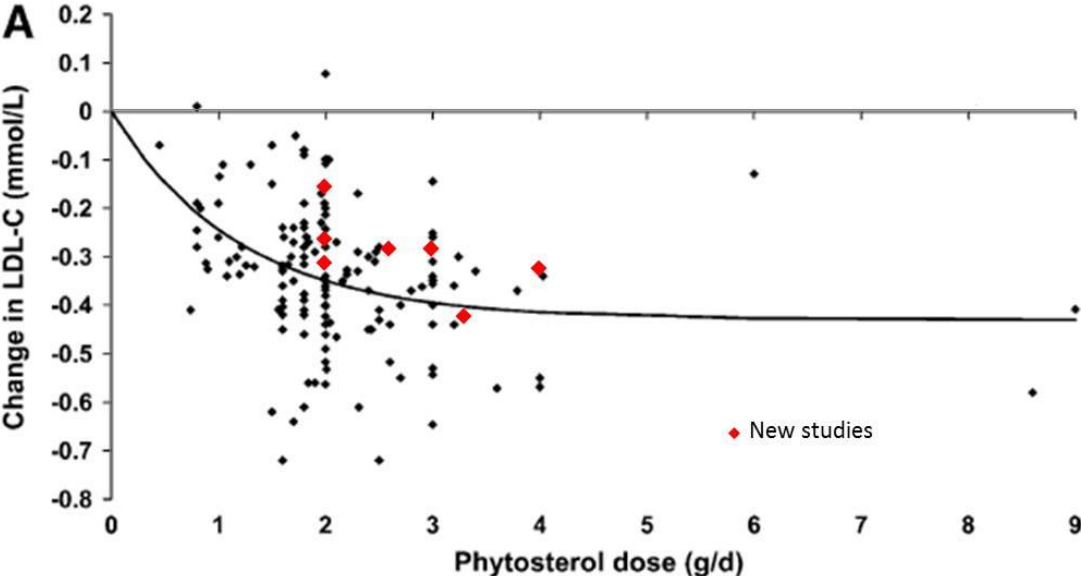


Figure 2 Overlay of the difference in LDL from the new studies reporting LDL cholesterol in mmol/L on Figure 3A of Demonty et al. (2009). Reproduced with permission.

Table 5 Changes in LDL cholesterol reported in studies included in the FSANZ update compared to two previously published meta-analyses

Study	Daily dose and type ¹	Duration (weeks)	Absolute change (mmol/L)	Relative Change ² (%)	Consistent with meta-analysis? ³
Demonty 2009 (meta-analysis)	2.2g (mixed, mean)	3 – 26	-0.34 (95%CI: -0.36, -0.31)	-8.8 (95% CI: -9.4, -8.3)	n.a.
Ras 2013 (meta-analysis)	1.6g (mixed, mean)	3 – 45	-0.33 (95%CI: -0.37, -0.30)	-8.5 (95% CI: -9.2, -7.7)	n.a.
Allen 2008	2.2g, sterol ester	4		-4.0	Y-
Banuls 2010	2.0g, sterol ester	13		-9.9	Y+
Banuls 2011	2.0g, sterol ester	13		-8.1	Y=
Baumgartner 2013	3.0g, sterol and stanol, esters	4	Sterol: -0.29 Stanol: -0.26	Sterol: -8.1 Stanol: -7.8	Y=
Buyuktuncer 2013	1.9g, stanol ester	4		-6.3 ³	Y-
Chen 2009	3.3g, sterol ester	3.1	Step 1 diet: -0.43 Western diet: -0.45		Y-
Dulalia 2011	2g, free sterol	8	Pineapple juice: -0.32 Orange juice: -0.28 (ns) Placebo: -0.26		Y=-
Gagliardi 2010	2.5g, sterol ester	5		-11.4 (median change, ns)	Y+
Goncalves 2007	2g, sterol, unclear	4.3		-8.7 (30d) Note: similar decrease observed with placebo	Y=
Gylling 2013	3g, stanol ester	26	-0.29	-10.2 ⁴	Y+
Hallikainen 2013	2.7g, stanol ester	4		-8.5 ¹ , -11.1 ⁴	Y=/+
Hernandez-Mijares 2011	2.0g, sterol ester	13		MetS(-ve): -10.5% Mets (+ve): no change	Y+ N
Khandelwal 2009	2.0g, sterol ester	4	-0.16 ⁵	-4.5 ⁵	Y- ⁴
Ruiu 2009	1.6g, sterol ester (=1g free sterol)	4		-4.3	Y-
Shaghghi 2014	2.0g, water-dispersible free sterols and sterol esters	4.1	Free sterols: -0.27 Sterol esters -0.25	Free sterols: -11.7 ⁴ Sterol esters: -11.64	Y+
Sialvera 2012	4.0g, sterol, unclear	8.7		-20.3	Y+
Soderholm 2012	2.0g for 2wk then 4.0g for 2wk, free sterols	4	-0.32	-10.4	Y+
Weidner 2008	2.6g, sterol ester (=1.6g free sterol)	8	-0.29	-6.8	Y-

¹Dose as reported as either free or esterified. In meta-analysis dose was presented as free sterol equivalents.

²Relative change was usually reported as change from baseline, not the difference in change between intervention and placebo groups, except where indicated.

³Y+; consistent with meta-analysis effect larger than 95%CI, Y-; consistent with meta-analysis but effect lower than 95%CI, Y=; within 95%CI of effect estimate from meta-analysis, N; not consistent with direction of effect estimate.

⁴Change adjusted for difference in control group

⁵Difference from ANCOVA analysis. Note, the unadjusted values showed a +0.01 mmol/L increase in LDL cholesterol from baseline to end of intervention with plant sterols.

MetS: metabolic syndrome

3.2.3 Quality assessment of individual studies

The risk of bias analysis for individual trials is presented in Appendix 3, and is summarised in Table 6. The overall body of new evidence was at low risk of selection, performance, detection, attrition and reporting bias. Eight of the 19 studies had a high risk of at least one type of bias. Blinding of outcome assessors was unclear for the majority of trials, and was classified as high risk for 3 trials which were described as 'single blind'. However, given the outcome of interest is measured objectively this is unlikely to affect the overall quality of these trials.

Publication bias was not formally assessed in the update. In the review by Demonty et al. (2009), publication bias was not observed. While, Ras et al. (2013) noted significant publication bias for serum phytosterol levels, it was not present for cholesterol outcomes. Comparison of results from recent trials with the effect estimates in the meta-analysis by Demonty et al. (2009), demonstrated an even distribution of results above, below or within the effect estimate and so does not suggest that the new data would change this conclusion.

Consideration of individual trials found that all had clearly stated objectives with adequate experimental design to assess the research question. Approximately half of the trials used a power calculation to determine sample size, although these calculations were not always performed for blood cholesterol outcomes (see Appendix 2). Confounding factors were identified in four trials (Allen et al. 2008; Khandelwal et al. 2009; Sialvera et al. 2012; Soderholm et al. 2012), with insufficient data in another two trials to fully assess confounding dietary factors (Dulalia et al. 2011; Shaghghi et al. 2014). Of the four trials with identified confounders, in two of these trials the confounding would bias towards the null and was therefore not further considered (Allen et al. 2008; Soderholm et al. 2012). Details of confounding factors are presented in Appendix 2.

Table 6 Summary of risk of bias analysis in studies included in review update (see Appendix 3 for details)

Reference	selection bias - randomisation	selection bias - allocation	Performance bias	detection bias	attrition bias	reporting bias	Other
Allen 2008	?	✓	✓	✓	✓	✓	✓
Banuls 2010	?	?	x	?	✓	✓	✓
Banuls 2011	?	?	x	?	✓	?	✓
Baumgartner 2013	✓	?	✓	?	✓	✓	✓
Buyuktuncer 2013	✓	?	✓	?	?	✓	✓
Chen 2009	?	?	✓	✓	✓	✓	✓
Dulalia 2011	✓	✓	✓	✓	✓	✓	✓
Gagliardi 2010	?	?	✓	x	x	✓	✓
Goncalves 2007	?	?	✓	?	?	✓	x
Gylling 2009	✓	?	✓	?	✓	✓	✓
Gylling 2013	✓	?	✓	✓	✓	✓	✓
Hallikainen 2013	✓	?	✓	?	✓	✓	✓
Hernandez-Mijares 2011	?	?	x	?	✓	?	✓
Khandelwal 2009	✓	✓	✓	✓	✓	✓	✓
Ruiu 2009	?	?	✓	x	✓	✓	✓
Shaghghi 2014	?	?	?	?	✓	✓	✓
Sialvera 2012	?	?	✓	x	✓	✓	x
Soderholm 2012	✓	?	✓	?	✓	✓	✓
Weidner 2008	?	?	✓	?	✓	x	✓

✓; low risk, ?; unclear risk, x; high risk

3.2.4 Outcome data

Reported changes in LDL cholesterol were extracted to enable comparison of these data with the effect estimates from the meta-analyses by Demonty et al. (2009) and Ras et al. (2013). LDL cholesterol concentrations were reported in all except one trial, although some trials reported only % change and did not give absolute change in mmol/L. As shown in Table 5, the majority of recently reported data is consistent with the effect estimates, with an even distribution of studies reporting a greater or lesser decrease in LDL cholesterol concentrations. Similarly, results for total cholesterol in the trials included in the FSANZ review update were generally consistent with the effect estimate reported by Ras et al. (2013) (see Table 7).

As summarised in Appendix 2, all except two trials reported a significant reduction in total and LDL cholesterol following phytosterol intake. Of these two trials, one reported only total cholesterol concentrations, which were significantly decreased (Gylling et al. 2009). The other trial found an 11.3% decrease in LDL cholesterol, but this was not significant (Gagliardi et al. 2010).

Two of the trials that reported significant decreases require caution in their interpretation. In one, a significant decrease in both total and LDL cholesterol concentrations was reported following adjustment for baseline cholesterol and other variables. However, the unadjusted data showed no effect of the phytosterol intervention on total and LDL cholesterol, while levels increased in the placebo group (Khandelwal et al. 2009). This interpretation of this study was confounded by a significantly lower baseline LDL cholesterol concentration in the

phytosterol group, despite randomisation. While the interpretation of this study is limited by this, it does not alter the overall body of evidence. In the other trial, significant decreases in LDL cholesterol were reported, but similar decreases were observed in the placebo control group (Dulalia et al. 2011).

Seven trials were assessed as being high quality, using the criteria of low risk of bias and no confounding factors (Allen et al. 2008; Gylling et al. 2009; Soderholm et al. 2012; Buyuktuncer et al. 2013; Gylling et al. 2013; Hallikainen et al. 2013; Baumgartner et al. 2013a). The outcome data of these trials were evenly distributed around the effect estimates from the meta-analyses by Demonty et al. (2009) and Ras et al. (2013). This is consistent with the dose-response analysis by Demonty et al. (2009) which did not find any covariate effect of study quality.

Table 7 Changes in total cholesterol reported in studies included in the FSA NZ update compared to the previously published meta-analysis

Study	Daily dose and type	Duration (weeks)	Absolute change (mmol/L)	Relative Change ¹ (%)	Consistency with meta-analysis ²
Ras 2013 (meta-analysis)	1.6g (mixed, mean)	3 – 45	-0.36 (95%CI: -0.40, -0.32)	-5.9 (95% CI: -6.5, -5.3)	n.a.
Allen 2008	2.2g, sterol	4	-0.14	-2.0	Y-
Banuls 2010	2.0g, sterol	13		-6.4	Y=
Banuls 2011	2.0g, sterol	13		-5.1	Y-
Baumgartner 2013	3.0g, both	4	Sterol: -0.30 Stanol: -0.29	Sterol: -5.3 Stanol: -5.3	Y=
Buyuktuncer 2013	1.9g, stanol	4		-4.6 ³	Y-
Chen 2009	3.3g, sterol ester	3.1	Step 1 diet: -0.48 Western diet: -0.49		Y+
Dulalia 2011	2g, sterol	8	Pineapple juice: -0.18 Orange juice: -0.24 (ns) Placebo: -0.18 (ns)		Y-
Gagliardi 2010	2.5g, sterol	5		-1.1	Y-
Goncalves 2007	2g, sterol	4.3		-6.7	Y+
Gylling 2009	2.13g, stanol 2.15g, sterol	52		Stanol: -4.2 ³ Sterol: -4.4 ³	Y-
Gylling 2013	3g, stanol	26	-0.20	-6.6	Y+
Hallikainen 2013	2.7g, stanol	4		-6.4	Y=
Hernandez-Mijares 2011	2.0g, sterol	13		MetS(-ve): -6.9% Mets (+ve): no change	Y+ N
Khandelwal 2009	2.0g, sterol	4	-0.17 ⁴	-3.2 ⁴	Y- ⁴
Ruiu 2009	1.6g, sterol ester (equivalent to 1g free sterol)	4		-2.4	Y-
Shaghghi 2014	2.0g, water-dispersible (WD) sterols and sterol esters	4.1	WD-sterols: -0.26 Sterol esters -0.18	WD-sterols: -7.7 ³ Sterol esters: -6.3 ³	Y+
Sialvera 2012	4.0g, sterol	8.7		-15.9	Y+
Soderholm 2012	2.0g for 2wk then 4.0g for 2wk, sterols	4		-6.5	Y=
Weidner 2008	2.6g, sterol ester (equivalent to 1.6g free sterol)	8	-0.26	-3.9	Y-

¹Relative change was usually reported as change from baseline, not the difference in change between intervention and placebo groups

²Y+; consistent with meta-analysis effect larger than 95%CI, Y-; consistent with meta-analysis but effect lower than 95%CI, Y=; within 95%CI of effect estimate from meta-analysis, N; not consistent with direction of effect estimate.

³Change adjusted for difference in control group

⁴Difference from ANCOVA analysis. Note, the unadjusted values showed a -0.04 mmol/L increase in LDL cholesterol from baseline to end of intervention with plant sterols.

MetS: metabolic syndrome

3.3 Participants with normal LDL cholesterol concentrations

The Demonty et al. (2009) review yielded 28 strata from studies conducted in people with normal LDL cholesterol concentrations (Figures 3 and 4) after excluding two studies with subjects using statin drugs (Goldberg et al. 2006; de Jong et al. 2008). One additional study (Myrie et al. 2012) was identified from Ras et al. (2013) and a further four strata from three studies from the FSANZ update (Dulalia et al, 2011; Khandewal et al. 2009; Soderholm et al. 2012).

The studies tested daily amounts of phytosterols ranging from 0.45 g to 9 g in people with normal cholesterol concentrations (Figure 3). Overall, phytosterols reduced concentration of LDL-C by an average -0.25 mmol/L (95% CI: -0.29, -0.21, $p < 0.00001$, Figure 3). The moderate degree of heterogeneity ($I^2=42\%$) might be related to the range of intake amounts and the effect being larger at higher amounts (Figure 4).

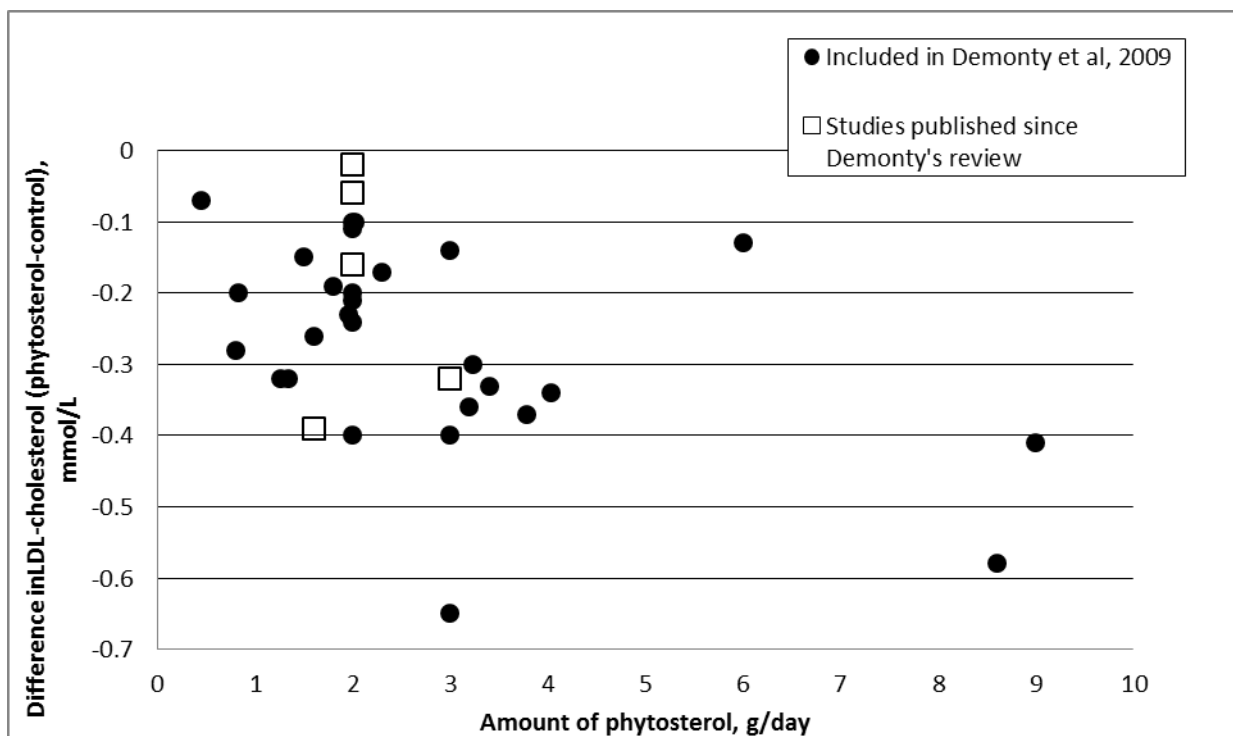


Figure 3 *Difference in LDL cholesterol concentration by phytosterol intake in people with normocholesterolaemia (mean baseline LDL cholesterol < 3.5 mmol/L)*

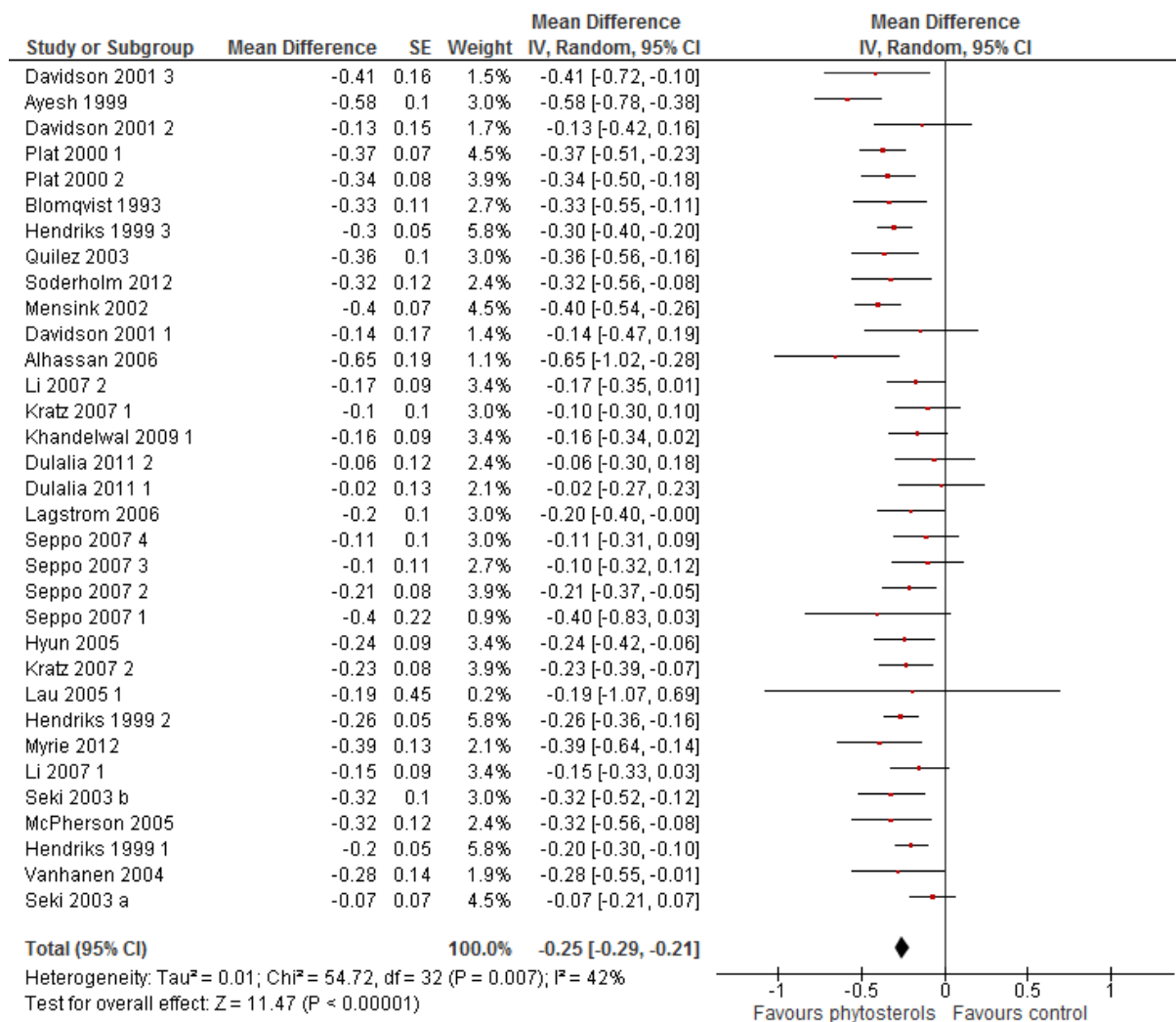


Figure 4 Forest plot for effects of phytosterols on LDL cholesterol concentration in people with normocholesterolaemia (mean baseline LDL cholesterol <3.5 mmol/L). The plot is ordered from highest daily intake amount of phytosterols at the top (9 g) to lowest daily amount at the bottom (0.45 g)

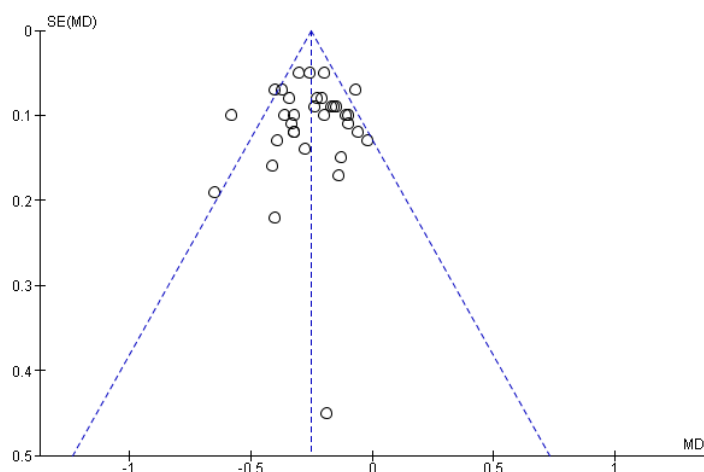


Figure 5 Funnel plot associated with the analysis shown in Figure 4

The funnel plot (Figure 5) might suggest that one or two studies with wide confidence intervals finding little or no effect might be missing from the dataset. However, given the size of the effect, the precision of the overall confidence interval and the number of studies already in the meta-analysis, this possible gap would not overturn the results.

3.4 Summary of new evidence

Nineteen published trials were included. These trials consistently reported a reduction in blood cholesterol concentrations following phytosterol consumption. The magnitude of effect was generally consistent with the effect estimates reported in the systematic reviews by Demonty et al. (2009) and Ras et al. (2013). Similar outcomes were reported from low and high quality trials. The relationship was present in studies conducted in people with normal LDL cholesterol concentrations.

4 Weight of evidence

4.1 Assessment of body of evidence

Together, the systematic reviews by Demonty et al. (2009) and Ras et al. (2013) included 172 strata reported in 106 publications. The FSANZ update identified another 19 publications reporting 22 strata. These 194 strata involved 9,128 participants. Their results indicated consumption of phytosterols reduced LDL cholesterol by approximately 0.33 mmol/L, or 8.5%. The data from recent trials are consistent with these effect estimates. Together, these data demonstrate a high degree of certainty in the relationship between phytosterol intake and reduced blood total and LDL cholesterol concentrations. Phytosterols reduce LDL cholesterol in people with normal cholesterol concentrations in studies using between 0.45-9 g/day.

4.1.1 Consistency and causality

More than 98% of the 194 reported strata reported a decrease in LDL cholesterol following consumption of phytosterols. This demonstrates the high degree of consistency between trials. Furthermore, a meta-analysis of interventions which administered phytosterols as a supplement reported very similar effect estimates to the Demonty and Ras reviews (Shaghghi et al. 2013). The effect is present in people with normal LDL cholesterol concentrations.

Randomised controlled trials are a strong study design for detecting causal relationships. As the trials included in the analysis were placebo controlled this provides a high degree of certainty in the causality of the relationship.

4.1.2 Plausibility

A number of mechanisms have been proposed to explain the cholesterol-lowering effects of phytosterols. Phytosterols and phytosterols are poorly absorbed from the intestine, at less than 10% and 1% the rate of cholesterol absorption, respectively. The structural similarities between phytosterols and cholesterol combined with their low absorption lead to competitive inhibition of cholesterol absorption. Specifically, phytosterols displace cholesterol from micelles leading to increased faecal cholesterol excretion. In addition, phytosterol intake has been shown to increase biliary cholesterol excretion (Racette et al. 2010). This is associated with a change in cholesterol partitioning with cholesterol being transported from blood into liver, to replace excreted bile acids.

4.2 Applicability to Australia and New Zealand

FSANZ has previously considered applications to add phytosterols to the food supply, as well as the addition of this food-health relationship to the list of pre-approved high level health claims in Standard 1.2.7. In doing so, it was considered that the relationship was relevant and applicable to the Australian and New Zealand populations. In addition, the review by Demonty et al. (2009) included 19 strata with Australian participants. The recent evidence does not alter earlier conclusions regarding applicability.

4.2.1 Intake required for effect

For the pre-approved health claim for phytosterols and reduced blood cholesterol in Standard 1.2.7, the dietary context statement states 'Diet containing 2 g of phytosterols, phytosterols and their esters per day'. The current data support this intake level as being adequate to achieve the stated health effect. This daily dose is consistent with equivalent international health claims. It is the same intake level used by Health Canada, and is within the range authorised for use in the EU (1.5-2.4 g per day). The US health claim sets a lower daily intake, at 1.6 g vegetable oil sterol esters.

4.2.2 Target population

The majority of trials assessing the effects of phytosterols have been conducted in adult populations with high blood cholesterol concentrations. However, the FSANZ update also shows that the same pattern of effect is present in those studies in which mean baseline LDL cholesterol concentrations <3.5 mmol/L. Therefore, no adult target group was identified from this review.

The reviews were restricted to adult populations, except one study which included children with familial hypercholesterolaemia. Therefore, the evidence assessed base does not provide data to substantiate the relationship between phytosterol intake and blood cholesterol concentrations in children.

4.2.3 Food matrix

The Demonty et al. (2009) review compared a number of food matrices and found no difference in absolute changes between fat and non-fat foods, dairy and non-dairy foods or solid and liquid foods. More recently, a review detailed the efficacy of phytosterol consumption from a variety of food matrices (Cusack et al. 2013). This review noted the potential for additive effects on blood cholesterol concentrations, for example from incorporation into food sources with high mono- or poly-unsaturated fat contents. In addition, in the current update the included trials used a variety of food matrices, including bread, milk, margarine, yoghurt and soy-drinks.

While the evidence supports the efficacy of phytosterols in a variety of food matrices, it should be noted that addition of phytosterols to foods requires pre-approval in Australia and New Zealand. Addition of phytosterols to edible oil spreads, breakfast cereals, cheese, milk and yoghurt is currently permitted.

4.2.4 Extrapolation from supplements

The majority of studies tested foods which had had phytosterols added to them. As it was possible to test identical foods without phytosterols as the control, there is high certainty that phytosterols were the active component and no extrapolation from supplements is needed. Some studies testing phytosterol given as capsules were also included.

4.2.5 Adverse effects

FSANZ has previously assessed the safety of adding phytosterols to foods. The current assessment of recent data found no new evidence that would alter the conclusions of previous assessments.

5 Conclusion

The reviews by Demonty et al. (2009) and Ras et al. (2013) both concluded that consumption of phytosterols in food reduces blood LDL cholesterol concentrations by approximately 0.33 mmol/L, and total cholesterol concentrations by 0.36 mmol/L. Data published since these reviews has reported consistent findings. The analysis by FSANZ in people with normal cholesterol found a similar effect. This body of evidence establishes a high degree of certainty in the relationship between phytosterol intake and reduced blood total and LDL cholesterol levels. We conclude that the relationship between phytosterol intake and 'reduced blood cholesterol concentrations' remains current and is applicable in adults with normal cholesterol concentrations as well as those with elevated cholesterol concentrations at the daily intake of 2 g phytosterol equivalents as stated in Standard 1.2.7.

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Appendix 1 – Search strategies for previous reviews and FSANZ update

Demonty et al. 2009

Searched in July 2007

Search terms:

Phytosterols (MeSH) or lipids (MeSH) or cholesterol (MeSH) or (plant sterol* or plant stanol* or phytosterol* or phytostanol* or sitosterol* or sitostanol* or campesterol* or campestanol* or stigmasterol* or brassicasterol*)
and (cholesterol* or blood lipid* or LDL cholesterol* or HDL cholesterol* or triglyceride*)

Searches were limited to human and clinical trials when possible.

Databases searched:

MEDLINE, Cab Abstracts, Biological Abstracts, Web of Science, and the Cochrane Library

Ras et al. 2013

Searched to June 2012

Search terms:

Medical Subject Heading 'phytosterols' and the search terms 'plant sterol* or phytosterol* or sitosterol* or campesterol* or stigmasterol* or brassicasterol*' and 'blood* or plasma or serum'

Databases searched:

Medline, Embase, Cab Abstracts, Food Science & Technology Abstracts, HCA Plus and Biosis

FSANZ update 2014

PubMed

Searched 7/4/14

Search terms:

phytosterols[MeSH Terms] OR plant sterol* OR plant stanol* OR phytosterol* OR phytostanol* OR sitosterol* OR sitostanol* OR campesterol* OR campestanol* OR stigmasterol* OR brassicasterol*

AND

blood* or plasma or serum

AND

"randomized controlled trial"[Publication Type] OR "controlled clinical trial"[Publication Type] OR randomi*ed[Title/Abstract] OR placebo[Title/Abstract] OR randomly[Title/Abstract] OR trial[Title/Abstract] OR groups[Title/Abstract]

NOT

animals[MeSH Terms] NOT "humans"[MeSH Terms]

Filters: publication date June 1 2012 onwards

EMBASE

Searched 10/4/14

Search terms:

1. phytosterol.sh or plant sterol\$.mp. or plant stanol\$.mp. or phytosterol\$.mp. or phytostanol\$.mp. or sitosterol\$.mp. or sitostanol\$.mp. or campesterol\$.mp. or campestanol\$.mp. or stigmasterol\$.mp. or brassicasterol\$.mp.
2. (blood\$ or plasma or serum).mp.
3. (randomized controlled trial or controlled clinical trial).sh.
4. (randomi?ed or placebo or randomly or trial or groups).ti,ab.
5. 3 or 4
6. 1 and 5
7. 6 and human/
8. limit 7 to yr="2012 -Current"

[mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword]

Food Science and Technology Abstracts

Searched 31/3/14

Search terms:

phytosterol* or plant sterol* or plant stanol* or phytostanol* or sitosterol* OR sitostanol* OR campesterol* OR campestanol* OR stigmasterol* OR brassicasterol*
AND
blood* or serum or plasma

limits: 2012-2014

Cochrane CENTRAL

Searched 31/3/14

- #1 MeSH descriptor: [Phytosterols] explode all trees
- #2 plant sterol\$
- #3 plant stanol\$
- #4 phytosterol\$
- #5 sitosterol\$
- #6 campesterol\$
- #7 stigmasterol\$
- #8 brassicasterol\$
- #9 blood\$ or plasma or serum
- #10 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8
- #11 #9 and #10 Publication Date from 2012 to 2014

International Clinical Trials Registry Platform

Searched 24/4/14

Search terms:

plant sterol or plant stanol or phytosterol or phytostanol

Appendix 2 – Included Studies in FSANZ update

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Allen 2008	Cross-over	Assess effects of consuming flavanols containing chocolate bar with or without phytosterols on blood lipids, blood pressure & inflammation.	49 adults with hypercholesterolaemia were randomised (24-70y, 65% male). 44 completed trial.	Subjects consumed 2x dark chocolate bars daily within 30m of a meal. Bars contained 180mg cocoa flavanols, and intervention bars also had 1.1g canola sterol esters per bar. 2wk run in on AHA diet, then 2x 4wk intervention periods with no wash out.	Dietary intake by 3d food records every 2wk. Compliance assessed by weekly interview. Blood lipid measured in certified laboratory and LDL measured directly. RMANOVA and <i>t</i> -test analysis.	Minimised by cross over design. Significantly high intake of saturated fat and cholesterol in PS arm, but this would bias towards the null.	Total and LDL cholesterol were significantly decreased in the PS compared to control arm. No significant change in HDL or triglycerides.	Power calculation performed for LDL-C. Industry and university funded study
Bañuls 2010	Parallel	To test the effects of long-term intake of PS in low-fat milk on cardiovascular risk factors and oxidative stress	40 subjects with untreated moderate hypercholesterolaemia (25-30% male, 24-69y). All completed trial.	Participants consumed a standard healthy diet for 3mo then were randomised to continue diet for 3mo with 500mL per day regular or PS-enriched low-fat milk. Test milk contained 2g PS per 500mL as esterified vegetable oil sterols.	Compliance by interview and return of unused products. Dietary intake by 3d food records and 24h food recall with dietitian. Blood lipid measured enzymatically with LDL calculated. RMANOVA and <i>t</i> -test analysis.	Subjects well matched post-randomisation. Dietary intake was reported to comply with guidelines.	Significant reduction in total and LDL cholesterol in PS group, which was additional to the reductions observed in the diet only phase. No significant change in HDL cholesterol.	Power calculation performed for LDL-C. Government funded.
Bañuls 2011	Parallel	To determine the effect of Apolipoprotein E genotype on blood lipid response to consuming PS in low-fat milk.	81 participants with mild to moderate hypercholesterolaemia were randomised (42% male, 18-76y). 75 completed the trial.	Same intervention as Bañuls 2010	Same methods as Bañuls 2010	Dietary intake for study not reported, but reference to an earlier trial was included.	Significant reduction in total and LDL cholesterol in PS group, with no effect of genotype.	Industry and government funded.

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Baumgartner 2013	Cross-over	To compare the effects of plant sterols with stanols on oxysterol levels	47 healthy subjects (38% male, 18-70y), 43 completed study. Mean baseline LDL-C concentrations was 3.5 mmol/L.	Subjects consumed 20g margarine per day containing either no PS, 3g plant sterols or 3g plant stanols. PS were esterified with rapeseed oil fatty acids. 3x 4wk periods with 4wk washout.	Dietary intake by validated FFQ at end of each intervention period. Compliance by return of unused product. Blood lipid measured enzymatically with LDL calculated. ANOVA analysis with Bonferroni post-hoc testing.	Minimised by cross-over design and similarity of dietary intakes between intervention periods.	Significant decrease in total and LDL cholesterol in both sterol and stanol periods. No differences between sterol and stanol period. No change in HDL levels.	Power calculation performed for oxysterol levels. Government funded.
Buyuktuncer 2013	Parallel	To assess the effects of plant stanol intake in yoghurt in a Turkish population	70 subjects with untreated mild to moderate hypercholesterolaemia (27% male, 23-65y). 35 participants in each group, withdrawals not reported.	Participants consumed 115g yogurt with or without 1.9g plant stanol esters with lunch each day. 2wk run-in, 4wk intervention.	Dietary intake by 3d diet diary in run-in and week 4. Compliance by patient interview and return of uneaten product. Blood lipids by standard techniques with LDL calculated. Analysis by general linear model repeated measures procedure.	Groups well matched at baseline and dietary intakes similar during intervention.	Significant decrease in total and LDL cholesterol. No significant change in HDL cholesterol.	Industry funded.

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Chen 2009	Cross-over	To assess the effects of 3 daily serves of phytosterols in two different background diets.	14 men and 9 postmenopausal women were randomised. Average age was 52y, and mean baseline cholesterol concentrations were mildly elevated. Data for one male subject excluded for suspected noncompliance.	All meals provided to participants. Diets were either a typical American diet or a Step 1 diet (low saturated fat and cholesterol), with or without 3.3g plant sterols per day. Plant sterols were consumed in margarine or salad dressing at 3 meals. 4x 4wk periods with no washout. End of intervention blood was drawn after 22 and 24d.	All meals provided, with weekday breakfast and dinner consumed on site. Blood lipids measured enzymatically with LDL calculated. Analysis by mixed effects model for repeated measures, with baseline values used as a covariate.	Minimised by cross-over design and provision of all meals and snacks	Total and LDL cholesterol concentrations were significantly reduced with plant sterol intake in both background diets. There was no interaction between diet and plant sterol intake.	Decreased plasma tocopherols and carotenoids reported with plant sterol intake. Government and industry funded.
Dulalia 2011	Parallel	To assess the LDL-C-lowering efficacy of 2 flavoured juices containing phytosterols	90 Filipino adults with LDL-C between 2.8 & 4.8mmol/L not taking cholesterol that affect cholesterol concentrations were randomised (unclear gender distribution, age 25-60y). 89 completed trial.	Subjects randomised to receive phytosterol in orange or pineapple juice, or placebo (water coloured and flavoured to be similar to test products). 8wk intervention with blood lipid measured at 4, 6, 8 and 12wks (after wash out period)	Compliance and dietary intake by daily food diary. Unclear method of blood lipid analysis. RMANOVA analysis.	Groups stated to be well matched at baseline. Dietary intakes not assessed. Per protocol analysis used, but unclear if 1 withdrawal was only exclusion from analysis.	Significant decrease in LDL-C at 4, 6 and 8wk for pineapple juice group, but triglyceride levels were increased in this group. LDL-C decreased significantly at 6wk only in orange juice group with no other significant changes in blood lipids. At 8wk there was a significant reduction in LDL in placebo group, and significant increase in triglycerides at all time points.	No difference in adverse effects between placebo and intervention groups. Reported adverse effects included headache and nausea, with frequency decreasing during trial. Funding appears to be from industry.

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Gagliardi 2010	Parallel	To compare the effects of butter, <i>trans</i> fat free margarine and plant sterol enriched margarine on blood lipids, lipoproteins, inflammation and endothelial function.	75 subjects with metabolic syndrome were randomised, 53 completed the trial (37% male, mean age 47y).	Subjects continued regular diet and exercise and consumed either 18g butter, 36g <i>trans</i> fat free margarine or 30g plant sterol margarine per day. 5wk trial.	Compliance by bi-weekly telephone monitoring. Non-compliant subjects excluded. Dietary intake by 3x 1d food diaries. Blood lipids measured enzymatically, including LDL-C. RMANOVA	Margarine well matched on baseline clinical characteristics. Median saturated fat intake was higher in plant sterol group at baseline, but difference was not significant.	Changes reported as median not mean. No significant difference in total, LDL or HDL cholesterol, but there was a median decrease of 11.3% in LDL-C in the plant sterol group.	The butter group was not considered as the <i>trans</i> fat free margarine was considered the appropriate control for this review. Government funded study.
Goncalves 2007	Parallel	To test the efficacy and safety of phytosterols in milk in healthy and hypercholesterolaemic subjects	22 healthy (not considered in review) and 34 hypercholesterolaemic subjects (32% male, 40-72y) randomised.	Hypercholesterolaemic subjects received placebo or phytosterol enriched milk (2g per day) for 30d.	Compliance unclear. Dietary intake not assessed. Method of blood lipid analysis not detailed. Statistical analysis by Student's <i>t</i> -test only.	Not well controlled. Baseline characteristics not reported, except difference in gender distribution (20% male in control, 42% male in phytosterol group).	A significant reduction in total and LDL cholesterol is reported, but similar albeit non-significant reductions were observed in placebo group. Had the change between groups been compared it would be unlikely to be significant.	No adverse effects were reported from analysis of various blood parameters. The intervention in healthy subjects was not considered for the review as no placebo groups was used. Industry funded

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Gylling 2009	Parallel	To assess the effects of long term intake of plant sterols and stanol on blood lipids, and investigate interaction with genetic polymorphisms	297 subjects with hypercholesterolaemia randomised (46% male, 25-70y), 282 completed trial (96 control group, 93 stanol group, 93 sterol group)	Subjects replaced 25g fat intake per day with control, plant sterol (2.15g) or plant stanol (2.13g) spread. 12mo trial.	Compliance reported as good, but method unclear. Dietary intake assessed by 3d food records at baseline and end of intervention. Blood lipids by gas-liquid chromatography. RMANOVA with Bonferroni post-hoc testing.	Groups well matched at baseline. Some dietary changes with time, but similar between groups.	Total cholesterol decreased significantly in both the sterol and stanol groups. No difference between the sterol and stanol group.	Power calculation performed for total cholesterol. No effect of genotype observed for cholesterol outcomes. Industry funded.
Gylling 2013	Parallel	To assess the effects of long term plant stanol ester intake on surrogate markers of cardiovascular health	94 subjects randomised, 92 completed trial (38% male, 25-66y). 46 per group. No inclusion criteria for blood lipids, 72% with elevated cholesterol at baseline.	Subjects replaced 20g daily fat intake with control or plant stanol spread. 6mo trial.	Compliance by serum sitosterol levels. Dietary intake by 3d food record at baseline and end of intervention. Blood lipids measured enzymatically. RMANOVA with Bonferroni post-hoc testing.	Groups generally well matched. Intervention group 46% male, control 30%.	Significant decrease in total and LDL cholesterol in plant stanol group.	Power calculation performed for LDL-C. Industry funded study.
Hallikainen 2013	Parallel	To assess the safety and efficacy of a once daily dose of plant stanol esters in a soy-based mini-drink.	61 subjects with mild to moderate hypercholesterolaemia randomised, 56 completed trial (20% male, 30-66y). 29 subjects in control group, 27 in plant stanol group with lipid data reported for 26.	Subjects consumed their regular diet with addition of placebo or plant stanol soy-based mini-drink after lunch or dinner. 2.8g plant stanols in test drink, mean of 2.7g stanols consumed each day. 2wk run-in followed by 4wk trial.	Compliance by daily study diary. Dietary intake by 2x 3d food records. Blood lipids measured by standardised methods. RMANOVA with Bonferroni post-hoc testing.	Groups generally well matched.	Significant decrease in total and LDL cholesterol in plant stanol group.	Adverse effects similar between groups. Industry funded study.

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Hernandez-Mijares 2011	Parallel	To compare the effects of consuming PS in low fat milk in subjects with and without metabolic syndrome	48 subjects with moderate hypercholesterolaemia (18-76y, 44% male, 24 with metabolic syndrome).	Same intervention as Bañuls 2010	Same methods as Bañuls 2010	Dietary intake for study not reported, but reference to an earlier trial was included.	Significant reduction in total and LDL cholesterol in subjects without metabolic syndrome. No significant change in cholesterol concentrations in subjects with metabolic syndrome.	Government funded.
Khandelwal 2009	Parallel	To assess the independent and interactive effects of plant sterols and fish oil on blood lipids	200 subjects with mildly elevated lipid levels (total cholesterol between 5.0-8.0mmol/L) were randomised, 178 completed trial (89% male, 35-55y). Note: 4-arm trial, 2 arms considered for review: control and plant sterol only arms.	Subjects consumed normal diet with placebo or plant sterol yoghurt drink, and placebo (safflower oil) or fish oil capsules. Control group (placebo yoghurt drink and safflower oil capsules) and plant sterol only group (2g per day plant sterol yogurt drink and safflower oil capsules) were considered. 2wk run-in prior to randomisation, then 4wk trial.	Compliance by daily recording of intake, return of unused and empty test products and phone call follow ups. Dietary intake by 2d, validated recall questionnaire. Blood lipids measured in certified laboratory. ANCOVA analysis of "quasi" intention-to-treat.	Despite randomisation the plant sterol group had significantly lower baseline LDL-C concentrations.	ANCOVA showed a significant decrease in total and LDL cholesterol. However, unadjusted data showed no effect of plant sterols on LDL-C in the test group, with an increase observed in the control group.	Power calculation performed for LDL-C and triglycerides. Funding source unclear.

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Ruiu 2009	Cross-over	To assess the effects of a daily single dose of plant sterols on lipoprotein metabolism	15 patients with non-familial hypercholesterolaemia were enrolled and completed the trial (67% male, mean age 54y)	Subjects consumed a controlled diet under the guidance of a dietitian, with a daily placebo or plant sterol yoghurt drink (100mL, with or without 1.6g plant sterol esters) consumed in the morning. 2x 4wk periods with a 3wk washout (diet continued during washout).	Food intake diary to monitor diet adherence, but intakes not quantified. Assessment of compliance unclear. Blood lipids measured enzymatically, LDL isolated by ultracentrifugation but quantification unclear. ANCOVA and <i>t</i> -test analysis.	Minimised by cross-over design and dietary advice.	Significant decrease in total and LDL cholesterol and increase in HDL cholesterol by <i>t</i> -test. Change in LDL and HDL cholesterol remained significant with ANCOVA.	Unclear funding source.
Shaghghi 2014	Cross-over	To compare the effects of a new formulation of water-dispersible (WD) plant sterols with that of plant sterol esters on blood lipids and fat soluble vitamins.	53 subjects with mild to moderate hypercholesterolaemia were recruited, 47 completed trial (53% male, 19-75y).	Subjects consumed their regular diet and attended the study centre once a day to consume 100g control or test yoghurt, containing 2g plant sterols (as esters or as WD-sterols). 3x 29d treatment periods, with 4wk wash-out.	Subjects consumed yoghurt in study centre. No dietary intake assessment. Blood lipids measured by automated techniques, with LDL calculated. Analysis by ANOVA with Dunnett's multiple comparison test.	Minimised by cross-over design. Lack of dietary assessment risks confounders not being identified.	Both sterol preparations significantly reduced total and LDL cholesterol concentrations.	No adverse effects observed. Power calculation performed for LDL-C. Industry funded study.

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Sialvera 2012	Parallel	To assess the effects of phytosterol consumption in subjects with metabolic syndrome	108 subjects (56% male, 30-65y) with metabolic syndrome were randomised to control (n=55) or intervention (n=53) group.	Subjects consumed their regular westernised diet with the addition of 2 yoghurt mini-drinks (placebo or test) per day after lunch and dinner. The test drinks provided 4g phytosterols per day. 2mo trial.	Compliance by fortnightly appointments with dietitian and weekly phone calls in intervention but not control group. Blood lipid measured enzymatically. Analysis by <i>t</i> -test.	Blood lipids tended to be higher in intervention group at baseline, but differences were not statistically assessed. Intervention, but not control, group were monitored closely by dietitian.	Significant decrease in total and LDL cholesterol concentrations. Small change in LDL-C in control group reported as significant, but this appears unlikely from the data.	Prior power calculations used to indicate adequate study participants. Funding source unclear.
Soderholm 2012	Parallel	To assess the cholesterol-lowering efficacy of phytosterol enriched rye bread.	68 subjects with cholesterol <6.5mmol/L were randomised, 63 completed the trial (25% male, mean age 35y in test group [n=32] and 37y in control group [n=31]).	6wk trial with 4 phases. Subjects consumed habitual diet for 1wk, then restricted cereal fibre intake for 1wk (baseline). In the intervention phases subjects consumed 99g rye bread for 2wk then 198g rye bread for a further 2wk. The test rye bread contained 2g free plant sterols in the low dose phase and 4g in the high dose phase.	Compliance by daily recording of cereal intake and serum markers of rye intake. Dietary intakes by 3d food records at baseline and end of low and high dose phase. Blood lipids measured enzymatically, with direct measurement of LDL-C after ultracentrifugation. Analysis by <i>t</i> -test.	Saturated and trans fat intake lower in intervention group at baseline, but this would bias towards the null. Groups well matched for other parameters. High fibre intake matched between groups.	Significant reduction in total and LDL cholesterol concentrations. After low dose period the change in the intervention group was significantly different to the change in the control group. After the high dose period, total and LDL cholesterol were significantly lower than baseline in the intervention group.	Mixed funding source.

Reference	Study design	Objectives	Participants & sample size	Interventions	Methods	Confounders	Results	Notes
Weidner 2008	Parallel	To assess the lipid-lowering effects of plant sterols in a soy drink.	50 subjects with untreated moderate hypercholesterolaemia were randomised, 49 completed trial (38% male, 19-65y).	Subjects consumed 200mL soy drink each morning, with or without added plant sterols (2.6g sterol esters, equivalent to 1.6g free sterols). 2wk run-in then 8wk intervention. Dietary recommendations during run-in and trial to avoid plant sterol enriched foods.	Compliance by counting empty packets. Dietary intake by food survey at baseline and of intervention. Blood lipid analysis methods not reported, LDL-C calculated. Intention to treat analysis used.	Dietary intake analysis not reported. Groups generally well matched at baseline.	Significant reduction in total and LDL cholesterol. No change in HDL cholesterol or triglyceride levels.	Power calculation for LDL-C performed. No difference in adverse effects between groups. Funding source unclear.

Abbreviations used: AHA; American Heart Association, ANCOVA; analysis of covariance, ANOVA; analysis of variance, HDL; high density lipoprotein, LDL-C; low-density lipoprotein cholesterol, RMANOVA; repeated measures ANOVA

Appendix 3 – Risk of bias assessment

Reference	Random sequence generation (selection bias)		Allocation concealment (selection bias)		Blinding of participants and personnel (performance bias)		Blinding of outcome assessors (detection bias)		Incomplete outcome data (attrition bias) >20% = high		Selective reporting (reporting bias)		Other	
Allen 2008	?	Matched on age, total cholesterol, BMI, but method not described	low	Numbered codes, investigators blinded	low	Double-blind	low	Investigators blinded until after analysis	low	10% attrition	low	Expected outcomes reported	low	
Banuls 2010	?	Unclear method of randomisation	?	Not described	high	Not blinded	?	Not described	low	No attrition	low	Expected outcomes reported	low	
Banuls 2011	?	Unclear method of randomisation	?	Not described	high	Not blinded	?	Not described	low	7% attrition	?	Dietary intake data not reported	low	
Baumgartner 2013	low	Computer-generated sequence	?	Not described	low	Double-blind	?	Not described	low	9% attrition	low	Expected outcomes reported	low	
Buyuktuncer 2013	Low	Randomisation list in blocks	?	Not described	low	Double-blind	?	Not described	?	Not described	low	Expected outcomes reported	low	
Chen 2009	?	Unclear method of randomisation	?	Not described	low	Double-blind	low	Investigators blinded until after analysis	low	4% attrition	low	Expected outcomes reported	low	
Dulalia 2011	low	Computer-generated sequence	low	Opaque envelopes	low	Double-blind	low	Blinded	low	1% attrition	low	Expected outcomes reported	low	
Gagliardi 2010	?	Unclear method of randomisation	?	Not described	low	Participants but not personnel blinded	high	Not blinded	high	29% attrition	low	Expected outcomes reported	low	
Goncalves 2007	?	Unclear method of randomisation	?	Not described	low	Participants but not personnel blinded	?	Not described	?	Not reported	low	Expected outcomes reported	high	No control group for healthy subjects

Reference	Random sequence generation (selection bias)		Allocation concealment (selection bias)		Blinding of participants and personnel (performance bias)		Blinding of outcome assessors (detection bias)		Incomplete outcome data (attrition bias) >20% = high		Selective reporting (reporting bias)		Other	
	low		?	Not described	low		?	Not described	low		low		low	
Gylling 2009	low	Frequency matched for gender	?	Not described	low	Double-blind	?	Not described	low	5% attrition	low	Expected outcomes reported	low	
Gylling 2013	low	Computer-generated sequence	?	Not described	low	Double-blind	low	Blinded	low	2% attrition	low	Expected outcomes reported	low	
Hallikainen 2013	low	Performed by independent statistician	?	Not described	low	Double-blind	?	Not described	low	8% attrition	low	Expected outcomes reported	low	
Hernandez-Mijares 2011	?	Unclear method of randomisation	?	Not described	high	Not blinded	?	Not described	low	no attrition	?	Dietary intake data not reported	low	
Khandelwal 2009	low	Computer-generated sequence	low	Sealed envelopes	low	Double-blind	low	Blinded	low	11% attrition	low	Expected outcomes reported	high	Difference in baseline LDL-C between groups
Ruiu 2009	?	Unclear method of randomisation	?	Not described	low	Participants but not personnel blinded	high	Not blinded	low	No attrition	low	Expected outcomes reported	low	
Shaghghi 2014	?	Unclear method of randomisation	?	Not described	?	Not described	?	Not described	low	11% attrition	low	Expected outcomes reported	low	
Sialvera 2012	?	Unclear method of randomisation	?	Not described	low	Participants but not personnel blinded	high	Not blinded	low	No attrition	low	Expected outcomes reported	high	Difference in protocol between arms.
Soderholm 2012	low	Stratified for total chol. and gender	?	Not described	low	Double-blind	?	Not described	low	7% attrition	low	Expected outcomes reported	low	
Weidner 2008	?	Unclear method of randomisation	?	Not described	low	Double-blind	?	Not described	low	2% attrition	high	Dietary intake data not reported	low	

Appendix 4 – GRADE summary of findings table

GRADE evidence profile of FSANZ’s updated systematic review

Question: What is the effect of phytosterol intake on blood cholesterol concentrations in adults?

Source: FSANZ update of Demonty et al. 2009 and Ras et al 2013 systematic reviews.

Quality Assessment of body of evidence								Effect	Quality (degree of certainty in relationship)	
Number of studies	Particip ants	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Considerations	Mean difference mmol/L (95% CI)		
Reduction in LDL cholesterol (from Demonty 2009)										
84	6805	RCTs	Low risk ¹	none	none	none	none	-0.34 (-0.36, -0.31)	⊕⊕⊕⊕ High	
Reduction in LDL cholesterol (from Ras 2013)										
41 ²	2084 ²	RCTs	Low risk ³	none	none	none	none	-0.33 (-0.37, -0.30)		
Reduction in LDL cholesterol (identified in 2014 FSANZ update)										
18	1111	RCTs	Low risk	none	none	none	New evidence is consistent with earlier reviews.	Not calculated. Range: -0.16 to -0.45 -4% to -20%	⊕⊕⊕⊕ High	
Reduction in total cholesterol (from Ras 2013)										
41	2084	RCTs	Low risk ³	none	none	none	none	-0.36 (-0.40, -0.32)		
Reduction in LDL cholesterol in normocholesterolaemic people (all studies,)										
16	2446	RCTs	Low risk	none	none	none	New evidence is consistent with earlier reviews.	-0.25 (-0.29; -0.21)	⊕⊕⊕⊕ High	

¹Covariate analysis found no difference between low and high quality studies or those with well or poorly randomised subjects

²18 trials were also included in Demonty et al. 2009, with 1169 participants in common

³Risk of bias analysis was not performed, but due to similarity in trials with Demonty et al. and requirement for a placebo-controlled study design, an assumption of low risk was made