Systematic review of the evidence for relationships between saturated, *cis* monounsaturated, *cis* polyunsaturated fatty acids and selected individual fatty acids, and blood cholesterol concentration

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Executive Summary

Does saturated fatty acid intake affect blood cholesterol concentration?	
Food health relationship	Decreased saturated fatty acid intake decreases blood total cholesterol and LDL cholesterol concentrations
degree of certainty (GRADE rating)	For isoenergetic replacement by carbohydrate: Total and LDL blood cholesterol concentrations: ⊕⊕⊕⊕ High For isoenergetic replacement by mono-unsaturated fatty acids: Total and LDL blood cholesterol concentrations: ⊕⊕⊕⊕ High For isoenergetic replacement by poly-unsaturated fatty acids with 18 carbon atoms: Total and LDL blood cholesterol concentrations: ⊕⊕⊕⊕ High

Does replacing saturated fatty acids with unsaturated fatty acids affect blood cholesterol concentration?	
Food health relationship	Replacement of saturated fatty acids with polyunsaturated and/or monounsaturated fatty acids decreases blood total cholesterol and LDL cholesterol concentrations
degree of certainty (GRADE rating)	For isoenergetic replacement by mono-unsaturated fatty acids: Total and LDL blood cholesterol concentrations: ⊕⊕⊕⊕ High For isoenergetic replacement by poly-unsaturated fatty acids with 18 carbon atoms: Total and LDL blood cholesterol concentrations: ⊕⊕⊕⊕ High

Do linoleic or α-linolenic acid intakes affect blood cholesterol concentration?	
Food health relationship	Increased intake of linoleic acid decreases blood total cholesterol and LDL cholesterol concentrations
degree of certainty (GRADE rating)	For isoenergetic replacement of carbohydrate: Total and LDL blood cholesterol concentrations: ⊕⊕⊕⊕ High
Food health relationship	Increased intake of α-linolenic acid decreases blood total cholesterol and LDL cholesterol concentrations
degree of certainty (GRADE rating)	For isoenergetic replacement of carbohydrate: Total and LDL blood cholesterol concentrations: ⊕⊕⊕⊕ High

Component	Notes
Body of evidence	This review covers a set of relationships regarding alterations to the type of fat consumed and the subsequent effect on blood cholesterol concentrations. An existing systematic review published in 2003 was updated to January 2014 and 25 more recently published randomised controlled trials (RCTs) were added to the existing review. This resulted in 74 studies (177 different diet strata) in the updated review. Multiple regression analysis estimated the effect of increasing or decreasing 1% of energy from the fatty acid classes or individual fatty acids of interest while holding total energy intake constant and controlling for the intake of other fats.
Consistency	For the classes of fats, the addition of more recently published studies had little effect on the results of the existing review. The RCTs showed that decreases in saturated fat with isoenergetic replacement by unsaturated fat (poly- or mono-unsaturated fat) decreased blood total-cholesterol (total-C) and LDL-cholesterol (LDL-C). This finding was consistent when the data were divided according to baseline cholesterol concentration, funding source, or period of publication and when studies using liquid formula diets as the source of nutrition were excluded. Isoenergetic replacement of saturated fatty acids by carbohydrate also reduced total-C and LDL-C concentrations, as did isoenergetic replacement of carbohydrate by the individual polyunsaturated fatty acids, linoleic and α-linolenic acids.
Causality	Randomised controlled trials are a strong study design for causality. Studies were included only if subjects were provided with all or most of their food. Other criteria included equality of dietary cholesterol content of test diets within a trial. These criteria increase the certainty that the results were not due to differential adherence to the allocated diet or other differences in intake. The isoenergetic exchange analysis means the results are not due to concurrent changes in body weight. Therefore the regression results support a causal link for the relationships assessed. The results were found in studies conducted in people with normal and elevated cholesterol concentrations.
Plausibility	The plausibility of the food-health relationships is supported by a substantial body of evidence from animal and human studies. For example, saturated fatty acids increase plasma LDL-C by increasing LDL formation and by decreasing LDL turnover, while the lowering of plasma LDL-C observed with PUFA is likely due to redistribution of cholesterol between plasma and tissue pools and upregulation of the LDL receptor.

The purpose of the review was to assess the currency of a pre-approved high level health claim relationship that decreasing saturated fatty acid intake decreases total-C and LDL-C concentrations in the blood. It also examined whether the relationships underpinning three claims authorised in the European Union (EU) relating to intake of unsaturated fats could be substantiated within the Australia New Zealand health claims framework. In doing this review, FSANZ has followed the mandatory requirements of Part 3 of the FSANZ *Application Handbook* and of Schedule 6 – Required elements of a systematic review in the *Australia New Zealand Food Standards Code.*

A suitable existing systematic review published in 2003 was identified and was updated for FSANZ by the original author. The search was performed in January 2014 and yielded 25 studies published since the literature search performed for the existing review, giving a total of 74 studies in the update. To increase certainty about the composition of the diets being tested and to remove the influences of imperfect adherence and intake reporting by subjects,

only studies which provided virtually all of the dietary intake to subjects were included. Studies which relied on subjects to follow dietary instructions and choose their own diets were excluded. Most included studies used a cross-over design and the test fats or oils were used as ingredients in food preparation. Most studies were at least single blind. The included studies were considered to be high quality. The studies were conducted over more than 40 years in a range of countries and with different types of oils, including some of the recently developed oils such as high oleic acid oils. The cholesterol lowering effect has been maintained across time and also within the sub-group analyses that were performed. The results did not vary by baseline blood cholesterol concentration and so apply to people with normal cholesterol concentrations.

The regression analysis found that replacing 1% of energy from saturated fatty acids (SFA) with the same amount of energy from carbohydrate decreased LDL-C concentration by 0.036 mmol/L. The decrease in LDL-C was similar when SFA were replaced by monounsaturated fatty acids (MUFA; 0.033 mmol/L) and higher when replaced by polyunsaturated fatty acids (PUFA; 0.042 mmol/L). These effects were statistically significant. The decreases in total-C concentrations were larger than for LDL-C concentration and also statistically significant. These results do not alter the conclusions drawn from the existing review conducted in 2003. The results for PUFA are based on studies which altered the amounts of the 18-carbon PUFAs, that is, linoleic acid (LA) and α-linolenic acid (ALA), and contained little of the other PUFA. Therefore, the data do not allow conclusions to be drawn regarding the effects of other PUFA such as arachidonic acid, eicosapentaenoic acid or docosahexaenoic acid on blood cholesterol concentrations. In addition, the studies of SFA did not examine the effects of altering the intake of short and medium chain saturated fatty acids. Replacing 1% energy from carbohydrate with 1% energy from LA or ALA (the two major PUFA in the diet) also reduced total-C and LDL-C concentrations.

It is noted that an isoenergetic exchange of one type of fat for another (for example replacing SFA with PUFA) is the same as exchanging one gram of fat for one gram of another fat because all fats are assumed to have the same energy content. However, this is not true when carbohydrate and fat are exchanged for each other. One gram of carbohydrate has less than one half the energy content of one gram of any type of fat. Therefore replacing one gram of carbohydrate with one gram of fat would not be an isoenergetic exchange but an exchange which increases energy intake. Increase in energy intake compared to expenditure leads to increases in body weight, which could have its own effect on cholesterol concentrations. Only the situation where energy intake, and therefore body weight, remains constant has been assessed. The effect of increasing or losing body weight simultaneously with altering the macronutrient content of the diet has not been assessed because it would not be possible to unambiguously attribute an effect on cholesterol concentration to the alteration in macronutrient composition from that type of study.

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 for all relationships investigated. The relationships are substantiated in people with normal cholesterol concentrations and for the current level of fatty acid intakes in Australia and New Zealand.

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Supporting Document 1: Mensink R (2016) Updated systematic review examining the effect of fatty acids on serum lipids.

Abbreviations and definitions

AHS	2011-2 Australian Health Survey
ALA	α-linolenic acid, a polyunsaturated omega-3 fatty acid
BMI	body mass index
HDL-C	high density lipoprotein cholesterol
LA	linoleic acid, a polyunsaturated omega-6 fatty acid
LDL-C	low density lipoprotein cholesterol; usually calculated from the Friedewald equation
OA	oleic acid, a monounsaturated omega-9 fatty acid
total-C	sum of all cholesterol fractions in the blood
EFSA	European Food Safety Authority
%En	intake of a macronutrient expressed as the proportion of total energy intake consumed by the subject; fatty acids contain 37 kJ/g and carbohydrates contain 17 kJ/g
EU	European Union
RCTs	Randomised controlled trials
SFA	saturated fatty acids, with the empirical formula $C_nH_{2n}O_2$
TFA	*trans* fatty acids
MUFA	*cis* monounsaturated fatty acids
PUFA	polyunsaturated fatty acids in which all double bonds have the *cis* configuration
95% CI	95% confidence interval

Symbols used in tables:

SFA → MUFA	SFA is isoenergetically replaced by MUFA
SFA → PUFA	SFA is isoenergetically replaced by PUFA
SFA → Carb	SFA is isoenergetically replaced by carbohydrate
Carb → SFA	carbohydrate is isoenergetically replaced by SFA
Carb → MUFA	carbohydrate is isoenergetically replaced by MUFA
Carb → PUFA	carbohydrate is isoenergetically replaced by PUFA
Carb → LA	carbohydrate is isoenergetically replaced by LA
Carb → ALA	carbohydrate is isoenergetically replaced by ALA

1 Introduction

This review examines the currency of an existing pre-approved health claim relating to saturated fatty acids (SFA) and blood cholesterol concentrations that was in Standard 1.2.7 – Nutrition, Health and Related Claims upon gazettal. The review also considers whether several related claims concerning unsaturated fatty acids authorised in the European Union (EU) can be substantiated within the Australia New Zealand health claims framework.

The pre-approved claim in the Code

The effect of SFA on blood total cholesterol (total-C) and LDL cholesterol (LDL-C) (Table 1) was considered during the development of the Standard (FSANZ 2005) and a claim included in the *Australia New Zealand Food Standards Code* (the Code) after advice from the then Scientific Advisory Group. This advice was based on a commissioned review which concluded that 'A health claim relating to the association between saturated fatty acids and LDL cholesterol is undoubtedly justified though one claiming a direct link between saturated fatty acids and coronary heart disease is a little more difficult to justify, given some inconsistencies in the data' (Booker and Mann 2005). This followed the process specified by FSANZ at the time but was not a systematic review.

Table 1: Conditions for permitted high level health claims relating to saturated fatty acids in section S4-4 of the Australia New Zealand Food Standards Code

Column 1	Column 2	Column 3	Column 4	Column 5
Food or property of food	**Specific health effect**	**Relevant population**	**Context claim statements**	**Conditions**
Saturated fatty acids	Reduces total blood cholesterol or blood LDL cholesterol		Diet low in saturated fatty acids	The food must meet the conditions for making a nutrition content claim about low saturated fatty acids
Saturated and trans fatty acids	Reduces total blood cholesterol or blood LDL cholesterol		Diet low in saturated and trans fatty acids	The food must meet the conditions for making a nutrition content claim about low saturated and trans fatty acids

The review (Booker and Mann 2005) also noted:

> '… the extent of LDL cholesterol reduction achieved by lowering intake of saturated fatty acids is dependent upon the source of replacement energy. Replacing saturated fatty acids with polyunsaturated fatty acids would result in appreciably greater reductions in LDL cholesterol than replacement with either carbohydrate or monounsaturated fatty acids. Not replacing a reduction in saturated fatty acids, partially or totally, and resultant weight loss would also result in additional reduction of LDL cholesterol.'

The relationship concerning the reduction in SFA intake was considered via three analyses referred to by Booker and Mann (2005): isoenergetic exchanges with either carbohydrate, MUFA or PUFA. Table 1 shows that there is a second pre-approved relationship in section S4-4 of the Code which gives 'saturated and trans fatty acids' as the food or property of food. FSANZ regards this as a combination of the above relationship for SFA and a separate relationship for *trans* fatty acids (TFA). The relationship with *trans* fatty acids is covered in a separate review (FSANZ 2015a).

Claims authorised in the European Union

In 2012, the EU authorised (European Commission 2012) three health claims referring to classes of unsaturated fatty acids or individual unsaturated fatty acids and 'maintenance of normal blood cholesterol levels'. Table 2 shows the claims together with the amounts of fat specified to qualify for the claim.

Table 2: Claims relating to fatty acids and blood cholesterol concentrations authorised by the European Union under Article 13(1) in 2012[1]

Nutrient, substance, food or food category	Claim	Conditions of use of the claim
Monounsaturated and/or polyunsaturated fatty acids	Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels [MUFA and PUFA are unsaturated fats]	The claim may be used only for food which is high in unsaturated fatty acids, as referred to in the claim HIGH UNSATURATED FAT[2] as listed in the Annex to Regulation (EC) No 1924/2006.
Alpha-linolenic acid (ALA)	ALA contributes to the maintenance of normal blood cholesterol levels	The claim may be used only for food which is at least a source of ALA as referred to in the claim SOURCE OF OMEGA 3 FATTY ACIDS[3] as listed in the Annex to Regulation (EC) No 1924/2006. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA.
Linoleic acid (LA)	Linoleic acid contributes to the maintenance of normal blood cholesterol levels	The claim may be used only for a food which provides at least 1.5 g of linoleic acid (LA) per 100 g and per 100 kcal. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 10 g of LA.

The EU claim (Table 2) for 'monounsaturated and/or polyunsaturated fatty acids' covers three separate claims (all SFA replacement claims): one for MUFA, the second for PUFA and the third for a mixture of MUFA and PUFA. In examining the evidence to support these claims, the European Food Safety Authority (EFSA) Panel on Dietetics Products, Nutrition and Allergies opinion (EFSA 2011a) referred to effects on LDL-C concentration and a primary reference cited is the systematic review of Mensink et al. (2003).

Table 2 also shows two EU claims referring to increased intake of two 18-carbon atom PUFA: linoleic acid (LA) and α-linolenic acid (ALA) with no mention of replacement in the

[1] Source: Source: Commission Regulation (EU) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health Text with EEA relevance http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32012R0432 (Accessed 15 July 2016)

[2] "where at least 70% of the fatty acids present in the product derive from unsaturated fat under the condition that unsaturated fat provides more than 20% of energy of the product." (http://ec.europa.eu/food/safety/labelling_nutrition/claims/nutrition_claims/index_en.htm, accessed 25 November 2015)

[3] "where the product contains at least 0.3 g alpha-linolenic acid per 100g and per 100kcal" (http://ec.europa.eu/food/safety/labelling_nutrition/claims/nutrition_claims/index_en.htm, accessed 25 November 2015)

claim wording. The EFSA opinion related to LA (EFSA 2009b) refers to the systematic review of Mensink et al. (2003) as showing that 'Replacing 1% of energy from carbohydrates with PUFA reduced LDL-cholesterol' and to one study showing that the effect of LA and ALA are similar (Goyens and Mensink 2005). The EFSA opinion related to ALA cites these references and several other studies showing that LA and ALA have the same effect on lipoproteins (EFSA 2009a). This allowed a conclusion that the result for PUFA in Mensink et al. (2003) applied to both LA and ALA.

Update of an existing systematic review

FSANZ is considering whether permissions in the Code shown in Table 1 are current and whether the relationships which underpin the claims shown in Table 2 are substantiated. FSANZ considers that 'maintenance of normal blood cholesterol levels' is part of the wording specifications for an EU function claim under Article 13(1) whereas the evidence EFSA assessed were the changes in cholesterol concentrations when fatty acid intakes were varied. FSANZ notes that all the EFSA opinions discuss the effect on LDL-C concentration but do not always discuss the effect on total-C concentration.

The review by Mensink et al. (2003) is more than 10 years old. Newer studies are available, including studies which have tested edible oils containing higher amounts of ALA than was the case previously.

Professor Ronald Mensink, Professor of Molecular Nutrition, NUTRIM School of Nutrition and Translational Research in Metabolism at the University of Maastricht responded to the call for tenders in 2013 and proposed to update his 2003 systematic review (Mensink et al. 2003). This work was done contemporaneously with another, larger, review (Mensink 2016). The updated review is available in two reports (Supporting Document 1 and Mensink (2016)) which give partially overlapping analytical results based on the same dataset. Mensink (2016) describes overall analyses and focuses on SFA results of specific interest to WHO whereas Supporting Document 1 focuses on the analyses of interest to FSANZ, including results for ALA and LA.

The purpose of this report is to consider the evidence from Professor Mensink's updated systematic review in the context of the Australia New Zealand health claims framework.

1.1 Food / property of food

There are five foods or properties of food covered in this review: three classes of fatty acids (SFA, MUFA and PUFA) and two individual fatty acids (LA and ALA).

Dietary fatty acids are linear hydrocarbon chains with a methyl group at one end and a carboxyl group at the other. SFA have the empirical formula $C_nH_{2n}O_2$. In unsaturated fats, one or more of the single bonds between adjacent carbon atoms is replaced with a double bond. Most fatty acids found in biological systems have an even number of carbon atoms. They are named according to the number of carbon atoms and the location of the first double bond from the methyl end and whether the configuration of the double bond is *cis* or *trans*.

The following definitions are given in subsection 1.1.2–2(3) in Standard 1.1.2 – Definitions used throughout the Code

> **'*monounsaturated fatty acids*** means the total of cis-monounsaturated fatty acids'
>
> ***'polyunsaturated fatty acids*** means the total of polyunsaturated fatty acids with cis-cis-methylene interrupted double bonds'

'saturated fatty acids means the total of fatty acids containing no double bonds'

'*trans fatty acids* means the total of unsaturated fatty acids where one or more of the double bonds are in the trans configuration'.[4]

The definition of *trans* fatty acids in the Code includes conjugated double bond systems (double bonds separated by one single bond) as well as double bonds that are methylene-interrupted (double bonds separated by a CH_2 unit). To be labelled as a polyunsaturated fat, all double bonds must have the *cis* configuration. Consequently, fatty acids with, for example, one *trans* bond and one *cis* bond, are labelled as TFA.

Fatty acids with 20 or more carbon atoms are less common in the diet and are classed as long chain fatty acids and often considered separately from those with 18 or fewer carbon atoms. The major review underpinning the EFSA opinions (Mensink et al. 2003) specifically excluded studies with enhanced content of omega-3 long chain fats because they had been reviewed elsewhere. Therefore, the following definitions apply in this review:

- SFA refers to an intake of SFA that reflects the general diet; specifically studies that manipulate or test the intake of a single SFA fatty acid are excluded from consideration
- MUFA refers to an intake of MUFA that reflects the general diet. Oleic acid (OA) is the predominant type of MUFA in the diet
- PUFA refers to intakes of mixtures of PUFA which reflects the general diet. LA is the predominant type of PUFA in the diet. ALA is also present where the diet is specifically stated to include the longer chain omega-3 fatty acids.

LA is an 18 carbon fatty acid with two double bonds (C18:2, *cis*-9, *cis*-12). It is an omega-6 fatty acid. It can be converted to the long chain PUFA, arachidonic acid.

ALA is an 18 carbon atom fatty acid with three double bonds (C18:3, *cis*-9, *cis*-12, *cis*-15). It is an omega-3 fatty acid. Approximately 10% of dietary ALA is converted to the long chain PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the body.

Fatty acids yield energy in the body and so intake of fats is often described as the proportion of energy that they contribute rather than grams of fat. In the 2011-2 Australian Health Survey (AHS), average SFA intake was 28.0 g per day and this contributed 11.8% of energy intake (%En) in persons aged 2 years and over. MUFA contributed 11.7 %En (Table 3A). PUFA contributed 4.7 %En. As shown in Table 3A, this total included long chain omega-3 fatty acids. LA contributed 3.9 %En and ALA, 0.6 %En (Australian Bureau of Statistics 2014). Fatty acid intakes were a little higher in the 2008-9 New Zealand Adult Nutrition Survey for persons aged 15 years and older: 13.1 %En, 12.4 %En and 4.9 %En for total SFA, MUFA and PUFA respectively (Table 3B, University of Otago and Ministry of Health 2012). Note that the percentages are for triglycerides and, therefore, include the contribution of glycerol.

[4] https://www.legislation.gov.au/Series/F2015L00385

Table 3A: Mean intake of energy and fatty acids, and their contribution to total energy intake, 2011-2 Australian Health Survey (adapted from ABS 2014)

	19 and over				Total 2 years and over			
	Mean intake		Intake as % energy		Mean intake		Intake as % energy	
			Mean	RSE			Mean	RSE
Energy[a]	8672	kJ	-	-	8522	kJ	-	-
Total Fat[b]	73.8	g	30.9	0.2	72.8	g	30.9	0.2
Saturated fat	27.7	g	11.5	0.1	28.0	g	11.8	0.1
Trans fatty acids	1391	mg	0.6	0.0	1390	mg	0.6	0.0
Monounsaturated fat	28.4	g	11.8	0.1	27.7	g	11.7	0.1
Polyunsaturated fat	11.4	g	4.8	0.1	10.9	g	4.7	0.1
Linoleic acid	9.4	g	4.0	0.1	9.1	g	3.9	0.1
α-Linolenic acid	1.4	g	0.6	0.0	1.3	g	0.6	0.0
Total long chain omega 3 fatty acids	281.4	mg	-	-	248.9	mg	-	-

[a] Energy includes energy from dietary fibre.
[b] Components may not sum to the total
RSE Relative Standard Error
- Not reported/applicable

Table 3B: Mean intake of energy and fatty acids, and their contribution to total energy intake, 2008-9 New Zealand Adult Nutrition Survey (University of Otago and Ministry of Health 2012)

	Mean intake[a]		Intake as % energy[b]	
			Mean	95% CI
Energy	9103	kJ	-	-
Total Fat	83	g	33.7	(33.3–34.1)
Saturated fat	32.4	g	13.1	(12.9–13.3)
Monounsaturated fat	30.5	g	12.4	(12.2–12.6)
Polyunsaturated fat	11.7	g	4.9	(4.8–4.9)

[a] Usual daily intake. These data were adjusted for intra-individual variation using PC-SIDE (http://www.side.stat.iastate.edu/).
[b] These data were not adjusted for intra-individual variation because the only methods that have been developed for ratios use multiple day repeats. Percent energy from fat for each participant was calculated as the energy from fat (conversion factor = 37.7 kJ/g) divided by the total energy intake.
- Not reported
95% CI 95% confidence interval

1.2 Health effect

The health effect is reduced blood total-C or LDL-C concentration. Cholesterol is a sterol (modified steroid), and is an essential structural component of animal cell membranes, required for membrane permeability and fluidity. It is also a precursor for the biosynthesis of steroid hormones, bile acids and vitamin D. Cholesterol is typically produced to the greatest extent in the liver and is measured in serum.

Cholesterol is produced endogenously at a rate of approximately 1 g per day and around 300 mg per day is consumed in the diet by people aged 19 years and older (ABS 2014). Ingested cholesterol is esterified and is poorly absorbed. Furthermore, cholesterol biosynthesis is directly regulated by homeostatic mechanisms. Thus a higher intake from food leads to a net decrease in endogenous production and *vice versa*. In the blood, cholesterol is carried within amphiphilic protein complexes as it is only slightly soluble in water. Different lipoproteins (among them HDL (high density lipoprotein) and LDL (low density lipoprotein)) are targeted to different tissues in the blood via their different apolipoproteins. Cholesterol can be carried by all the different lipoproteins, and is only targeted to different tissues depending on the receptor binding of the lipoprotein carrying it.

LDL-C in the blood is oxidised and taken up by macrophages. These macrophages may become engorged and form foam cells, which are trapped in the walls of blood vessels and contribute to atherosclerotic plaque formation. HDL-C, on the other hand, is believed to transport cholesterol back to the liver for excretion or to other tissues that use cholesterol. Thus an increase in LDL-C is associated with serious medical problems, whereas an increase in HDL-C is generally associated with better health outcomes (Djousse and Gaziano 2009).

Hypercholesterolaemia is described in Australia as being total serum cholesterol concentrations above 5.5 mmol/l.[5] The normal range for LDL cholesterol is described as 2.0-3.4 mmol/L by some[6] and <3.5 mmol/L by others[7].

1.3 Proposed relationships

The following food health relationships have been assessed:

- decreased saturated fatty acid intake decreases blood total cholesterol and LDL cholesterol concentrations
- replacement of saturated fatty acids with polyunsaturated and/or monounsaturated fatty acids decreases blood total cholesterol and LDL cholesterol concentrations
- increased intake of linoleic acid decreases blood total cholesterol and LDL cholesterol concentrations
- increased intake of α-linolenic acid decreases blood total cholesterol and LDL cholesterol concentrations.

As noted above, the review being updated specifically excluded from its scope studies which focused on increasing long chain omega-3 PUFA intake.

HDL-cholesterol (HDL-C) concentration in blood is used in risk prediction algorithms in New Zealand (Ministry of Health 2013) and Australia (NVDPA 2012) because it, or its ratio to total-C, is predictive of heart disease. Therefore, the effect of the above fatty acid comparisons on HDL-C and total/HDL-C ratio were also examined to assess whether improvements in total-C and LDL-C concentrations were accompanied by an undesirable effect on HDL-C concentrations or total/HDL-C ratios.

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

[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.

2 Summary and critical appraisal of the existing systematic review

2.1 The importance of isoenergetic exchange when considering macronutrients

Fat is one type of macronutrient and individual fatty acids contribute to the energy content of the diet. If the energy content is not taken into account during analysis, then any changes seen with varying fatty acid intakes might be due to varying intakes of energy and the consequent effects on body weight (assuming energy expenditure does not change). In other words, any effects on cholesterol concentrations might be due to confounding by body weight changes. Therefore, to achieve a result that is independent of weight change and so can be attributed to the changes in fatty acid intake, analyses involving macronutrients need to hold energy constant and specify which macronutrients are replaced for each other.

Therefore the analyses to examine the food-health relationships listed above relating to decreased saturated fatty acids considered:

- decreased intake of SFA and isoenergetic replacement by carbohydrate and total-C and LDL-C concentrations
- decreased intake of SFA and isoenergetic replacement by MUFA and total-C and LDL-C concentrations
- decreased intake of SFA and isoenergetic replacement by PUFA and total-C and LDL-C concentrations.

The EU claim for replacing SFA with unsaturated fats (Table 2) refers to MUFA "and/or" PUFA. Two of the above analyses cover the fatty acids as separate entities and are relevant to examine the food-health relationships listed above, namely:

- decreased intake of SFA and isoenergetic replacement by MUFA and total-C and LDL-C concentrations
- decreased intake of SFA and isoenergetic replacement by PUFA and total-C and LDL-C concentrations.

FSANZ considers that assessment of "MUFA and PUFA" is covered by a combination of the two separate analyses.

The EFSA opinions relating to ALA and LA refer to exchanging carbohydrate for these fatty acids (EFSA 2009a; EFSA 2009b) even though this is not articulated in the EU claim wording. Therefore the following will be analysed:

To examine increased LA:

- increased intake of LA as isoenergetic replacement of carbohydrate and total-C and LDL-C concentrations.

To examine increased ALA:

- increased intake of ALA as isoenergetic replacement of carbohydrate and total-C and LDL-C concentrations.

It is noted that some of the above analyses hold total fat intake constant because one fatty acid is exchanged for another. In other analyses, total fat intake either decreases (in the case of SFA) or increases (in the case of LA and ALA) because carbohydrate is involved in the exchange. As noted later, the analyses for the three types of fatty acid were run in the same model so that the result for each fatty acid is also adjusted for the effects of the other fatty acids. For analyses involving LA and ALA, OA was also added to the analysis to control for this individual MUFA as it is the main source of MUFA in the diet. However, SFA was considered as a class, not as individual fatty acids, because this conforms to the wording of the claims.

Finally, because all fatty acid mixtures are assigned the same energy value, exchanging SFA for PUFA or MUFA involves a gram-for-gram exchange in intake. However, carbohydrate contains less than half the energy density of fat, and so an isoenergetic exchange of SFA for carbohydrate involves increasing carbohydrate intake by 2.25 g for every 1 g reduction in SFA.

2.2 Methods used in the existing review

The 2003 review by Mensink et al. was identified as a suitable review to update owing to the methods used to collate the literature and the analytical methods which examined an isoenergetic exchange of each class of fatty acid with carbohydrate while adjusting for the other classes of fat. It was an update of earlier work (Mensink and Katan 1992). The 2003 review also separated *cis* unsaturated fats from *trans* unsaturated fats. This review is referred to in a number of the EFSA opinions regarding fatty acids (EFSA 2009a; EFSA 2009b; EFSA 2011a; EFSA 2011b).

2.2.1 Study selection

The 2003 review was performed early in the development of systematic reviews and their reporting and does not give the level of detail that more recent reviews give about search criteria. The authors note that they used ‘computer assisted literature search’ for ‘original-research studies that were published in English between January 1970 and December 1998…We also scanned reference lists and performed hands-on searches of journals’ (Mensink et al. 2003)

2.2.2 Eligibility criteria

The eligibility criteria are summarised in Table 4. The criterion that the food intake had to be thoroughly controlled and described limited inclusion to trials which had been conducted in metabolic wards or other similar situations. Therefore this review excluded studies in which subjects might have used, for example, margarine instead of butter as part of their self-selected usual diets in a free-living situation. It also excluded studies which only supplied oils to the subjects to consume over the weekend but did not control food intake on the weekend (R Mensink, personal communication 2015).

The authors comment that studies focused on long-chain (20 or more carbon atoms) omega-3 PUFA were excluded a priori because a review of these was already available. Studies of medium chain triglycerides were excluded at the analytical stage because there were too few studies for statistical analysis.

Table 4: FSANZ's interpretation of the PICOTS criteria for study selection used by Mensink et al. (2003)

Population	Adults (> 17 y) without gross disturbances of lipid metabolism or diabetes
Intervention	Food intake thoroughly controlled and described, with dietary fatty acids as the sole variable; cholesterol intake had to be constant (by adding dietary cholesterol as eggs, egg yolks or crystalline cholesterol to some trial arm diets if necessary because animal fats are high in dietary cholesterol); diets that focused on long chain polyunsaturated fatty acids were excluded
Comparator	As for the intervention arm but using a different intake of fatty acids
Outcomes	Total, LDL and HDL cholesterol, ratio of total to HDL cholesterol
Time	≥13 days
Study design	Parallel, crossover, or Latin-square designs included, before-and-after (sequential) designs that lacked a control group excluded; reported in an original article in English

2.3 Summary of results

The systematic review and regression analysis by Mensink et al. (2003) included 60 studies lasting 13–91 days in 1672 participants (70% male). Mean baseline total cholesterol among the studies which reported this (including those not relevant to the current review) ranged from 3.7 to 6.5 mmol/L and mean age ranged from 21 to 72 years. Almost all studies were conducted in North America or Europe. Only some of the analyses in the 2003 report are relevant to the current review and these analyses were conducted using 43 and 47 studies (102 or 114 strata) depending on which analysis is considered (Table 5). The results are reported as the effect on cholesterol concentrations when 1 %En from carbohydrate is replaced with the same amount of energy from the class of fats under consideration.

Replacing carbohydrate isoenergetically with SFA increased LDL-C concentration whereas replacing carbohydrate with either MUFA or PUFA decreased it. Replacing carbohydrate with PUFA had a larger effect than replacement with MUFA (Table 5). However, all three classes of fatty acid increased HDL-C concentration when compared to (that is when they replaced) carbohydrate; SFA having almost double the effect of PUFA on increasing HDL-C concentration. The effect of replacing carbohydrates with the various classes of fatty acids on total-C concentration was similar, but not identical, to that which would be predicted by the combined effects on LDL-C and HDL-C concentrations. As shown in Table 5, there are more strata in the analysis for the total-C than for LDL-C and HDL-C concentrations and this might explain small variations in the pattern of the results. All results, except the very small effect for the replacement of carbohydrate with MUFA on total-C concentration, were statistically significant.

Additional analyses examined duration of the study, inpatient/outpatient mode, and diet type (mixed solids or a liquid formula) but these were not important influences on the changes in cholesterol concentrations.

Mensink et al. (2003) do not present results for the exchange of classes of fats for each other. However, these effects can be determined from the results that are presented in Table 5. Replacing 1 %En SFA with MUFA would lead to a decrease in LDL-C of 0.041 mmol/L (-0.032 due to replacement of SFA with carbohydrate and -0.009 due to replacement of carbohydrate with MUFA). Replacement of 1 %En SFA with PUFA would decrease LDL-C concentration by 0.051 mmol/L. It can be inferred that these changes are statistically significant. Analyses of the effect of individual PUFA were not presented.

Table 5: Change in total, LDL and HDL cholesterol concentrations (mmol/L) predicted when 1% energy from carbohydrate is isoenergetically replaced by fats (Mensink et al. 2003)

Lipid	N strata; studies	Change in cholesterol (mmol/L) per percent of energy replaced		
		Carb → SFA	Carb → MUFA	Carb → PUFA
ΔTotal-cholesterol	114; 47	0.036	-0.006	-0.021
95% CI		(0.029, 0.043)	(-0.012, 0.000)	(-0.027, -0.015)
p		<0.001	0.061	<0.001
ΔLDL-cholesterol	102; 43	0.032	-0.009	-0.019
95% CI		(0.025, 0.039)	(-0.014, -0.003)	(-0.025, -0.013)
p		<0.001	0.004	<0.001
ΔHDL-cholesterol	102; 43	0.010	0.008	0.006
95% CI		(0.007, 0.013)	(0.005, 0.011)	(0.003, 0.009)
p		<0.001	<0.001	<0.001

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.
Carb → SFA: carbohydrate is replaced with SFA, etc.

2.4 Critical appraisal of the existing review

2.4.1 Study identification and selection

The literature was searched from 1970 onwards (which was to 1998 at that time). Although there were many studies that examined the effect of fats on cholesterol concentration in the decades prior to this, the older studies only examined effects on total-C concentrations. From approximately 1970, awareness arose that not all cholesterol sub-fractions had adverse health effects. The results in Table 5 are consistent with those of studies conducted prior to 1970 which showed that SFA had a stronger (and opposite) effect on total-C concentrations than PUFA (for example Keys et al. 1957, Hegsted et al. 1965; Keys et al. 1965). Therefore FSANZ considers that commencing the search date at 1970 does not lead to a bias in the results.

Further information about the search strategy used in the existing review, for example which database was searched, is provided in Supporting Document 1. Hand-searching of references in identified studies and selected journals was also done. Studies were included only if they were trial designs with control groups. Uncontrolled before-and-after studies were excluded which is considered appropriate. The minimum duration specified for trial inclusion is sufficient for changes in blood lipid outcomes to stabilise (Brussaard et al. 1982; Mensink and Katan 1987) and has been used by FSANZ previously when examining a relationship with cholesterol as the health effect (FSANZ 2015b). Not all trials that tested variations in dietary intake were included; trials were excluded if they did not control, or describe, all aspects of the dietary intake such that it was possible to attribute the effects to variation in fat rather than some other component such as variation in dietary cholesterol intake.

2.4.2 Assessment of bias

The quality of included studies was not specifically appraised in the review. Only studies that controlled dietary intake by supplying all or most of the food to the subjects, including replacement of dietary cholesterol intakes where it was lower owing to the use of vegetable oils in some test groups, were included in the 2003 review. As the authors note, failure to do this would mean that results might be attributable to variations in dietary cholesterol intake rather than fatty acid profile. This means that the information about fatty acid compositions being tested does not rely on the participants' understanding and implementation of dietary

instructions or ability to report what they ate. In this context the blinding (or otherwise) of subjects is less critical in that the subjects have relatively little choice in what they ate. Therefore, the included studies can be regarded as higher quality studies than excluded studies in which the subjects' food intake was not tightly controlled.

2.4.3 Data extraction and analysis

The measurement of total-C has been standardised for many years, but the same is not true of LDL-C. Mensink et al. (2003) calculated the concentration of LDL-C using the Friedewald[8] equation (this is normal laboratory practice) because various researchers had used different methods to measure LDL-C in their studies (Appendix 1). They converted all data to serum values even when reported as plasma values. To obtain the correct quantity of fatty acids, data reported as total fat was corrected to remove the glycerol component. In all of the included trials, fat intake in one arm was replaced for a different type of fat or by carbohydrate in other trial arms.

Regression models using PROC REG in SAS version 6 were conducted in which each trial was represented by a set of dummy variables to keep strata from each trial together and therefore exclude differences between studies such as age or body mass index (BMI) from confounding the results. The models included SFA, MUFA and PUFA so that the result for each class was controlled for the effects of the other classes. The coefficients estimated the effect of replacing 1 %En from carbohydrate with the fat of interest on absolute cholesterol concentrations in mmol/L.

Owing to the manipulation of the data to correct each stratum to a common comparison that allowed for different fatty acid composition it was not possible to calculate standard errors. A non-weighted regression was conducted in SAS with each study represented by a dummy variable. Regression diagnostics were examined. The residuals from the regression were examined for influential values, normality and heteroscedasticity and there was no suggestion of dependence in the data. Cook's Distance was used to check for influential outliers and one or two data points were excluded as a result because this normalised the data. The tolerance was low enough to exclude inappropriate inflation of regression coefficients due to collinearity in the data. The authors comment that they did not use a formal random effects model but that their approach would be similar to a random effects model because they could not separate out the sources of variance. The authors were particularly interested in the total-to-HDL cholesterol ratio and presented the scatterplot of the observed values to predicted values from their regression model. This had a correlation of 0.99 and the linearity in the regression is evident. Although this ratio is not the focus of current review, it indicates that the model has a good fit to the data for a closely related outcome and this increases confidence in the results.

This review did not usually calculate the weighted average difference (that is, meta-analysis). This is appropriate because each diet tested could vary in at least four possible ways (via proportions of carbohydrate, SFA, MUFA and PUFA) and so an overall average would not provide useful information about specific alterations in intake. Regression diagnostics were examined in several different ways and demonstrated that the model was a good fit to the data.

[8] Friedewald equation calculates LDL cholesterol using the following formula:
LDL cholesterol = total cholesterol – HDL cholesterol – (triglyceride/2.2) where all concentrations are in mmol/L

2.4.4 Data interpretation

As noted above, a critical factor in interpreting these results is to recognise that they relate to exchanging 1 %En from the fatty acid class with carbohydrate and not to an increase or decrease in the intake of the fatty acid class per se.

The 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 the effect is clear. Mozaffarian and Clarke (2009) came to a similar conclusion in their analysis.

2.5 Consideration of validity and strength of evidence

Of the analyses of interest (Section 2.1), only that of replacing SFA with carbohydrate is directly reported. The replacement of SFA with unsaturates (Table 2) can be inferred from the results of Mensink et al. (2003) shown in Table 5. The analyses presented are based on a large number of studies conducted over nearly three decades by different researchers in many countries.

Mensink et al. (2003) did not examine the effects of individual unsaturated fatty acids. They note an important caveat when interpreting their results for the PUFA class: 'in this report, total PUFAs may be considered to equal the omega-6 PUFAs with 18 carbon atoms (linoleic acid plus some α-linolenic acid)' (Mensink et al. 2003). In addition to the analysis of the class of SFA which reflected typical mixed diets, Mensink et al. (2003) provided analyses for some individual SFA.

FSANZ concludes that there is high confidence in the results presented and that this is a suitable existing systematic review to update. Exclusion of studies conducted prior to 1970 is not an important drawback. FSANZ notes that the results of Mensink et al. (2003) are consistent with the earlier Keys equation for total-C (Keys et al. 1965) further indicating that there is no bias related to time period. The inclusion criteria around the control over participants' consumption means that studies with less control of certain fat composition (which would be classed as lower quality studies) are excluded. Although Mensink et al. (2003) do not give all the analyses that are desired (for example replacing SFA with MUFA or PUFA or for increasing the intake of LA and ALA), it would be possible to conduct these analyses in the dataset or an updated version of the dataset.

3 Evaluation of new evidence

The primary author of the existing review updated it using the same methods. The result of the update is given in Supporting Document 1 and Mensink (2016). Analyses investigating effects on HDL-C concentration and total-C/HDL-C ratio were also assessed because undesirable changes in these parameters would be considered adverse effects. Some additional description of the methods and studies in the existing review was provided by Professor Mensink, as noted elsewhere in this report.

As noted above, the results presented in Supporting Document 1 derive from a concurrent project being carried out by Professor Mensink (Mensink 2016). Some additional information is included as part of his chosen output format, for example, information about the effect on triglyceride concentrations.

3.1 Methods

The PICOTS of the existing 2003 review shown in Table 4 were suitable for the update. Some additional detail is available regarding the inclusion/exclusion criteria in this report compared to the detail in the existing review (Mensink et al. 2003):

- protein and alcohol intake must be constant across study arms; daily dietary cholesterol intake had to differ by <100 mg across study arms
- fatty acids had to be exchanged for other fatty acids or carbohydrates
- studies involving concomitant interventions such as those resulting in body weight loss, were excluded
- studies which focused on hydrogenated long chain omega-3 fatty acids were excluded
- studies focusing on medium chain fatty acids (<10 carbon atoms) or one specific saturated fatty acid were excluded
- only studies with reported TFA intakes of <2 %En were included; if TFA content was not reported, the study was included
- studies had to report one or more of: serum/plasma total-C, LDL-C, HDL-C or triglyceride concentrations.

The 2003 review investigated the effect of *trans* fats on cholesterol concentrations in addition to the effect of other fatty acids. In the update, studies of diets enriched in *trans* fats which had been included in 2003 were excluded. In the update, the criteria for difference in dietary cholesterol intake between study arms were slightly different from the original review. Consequently several studies from the 2003 review were not included in the update while several others were added.

Analyses to support the food health relationships of interest to FSANZ are shown in Supporting Document 1. These are based on the subset of 74 studies from Professor Mensink's larger review (Mensink 2016) which were relevant to the specific relationships of interest to FSANZ. The same analytical methods were used in the existing review and the update (see Supporting Document 1 for additional detail). In summary, multiple regression weighted by the number of subjects for each datapoint was performed to determine the dose-response effect of a 1 %En replacement of either carbohydrate or SFA with the classes of fat or individual fatty acids listed above in Section 2.1. Specifically, to examine the effect of classes of fat, the model contained carbohydrate, SFA, MUFA and PUFA as exchanges for carbohydrate, and a different model with MUFA and PUFA as exchanges for SFA. To examine the effect of individual unsaturated fatty acids, the model contained SFA, OA, LA and ALA as exchanges for carbohydrate. Thus results for one macronutrient are adjusted for other macronutrients in the model. For these three sets of macronutrients, a separate model was run for each outcome (for example total-C, LDL-D). Dummy variables were used to code arms of each study to manage the multiple intervention arms in some studies. Results are presented for an unweighted analysis. Additional analyses in which the models were weighted by the number of participants or the inverse of the variance were conducted. As there was no important difference that would alter the interpretation of the results, the unweighted results were reported.

FSANZ described the degree of certainty of the results reported by Professor Mensink using the GRADE Framework (Guyatt et al. 2008) and assigned a rating to each analysis.

3.1.1 Subgroup analyses

The following subgroup analyses were carried out:

- populations with different baseline cholesterol concentrations
- form of the diet provided (liquid meal versus other)
- funding source (industry or not)
- year of publication.

In other reviews, FSANZ has applied the cut-off points of ≤5.5 mmol/L for total-C and <3.5 mmol/L for LDL-C to the mean reported baseline cholesterol concentrations to define studies which have been conducted in subjects with 'normal' cholesterol concentrations (FSANZ 2015b). In the current analysis, Professor Mensink dichotomised the studies at the medians of the baseline values reported in the studies. These were 4.45 mmol/L for total-C, 2.89 mmol/L for LDL-C and 0.97 mmol/L for HDL-C concentrations. This resulted in more than half the available data coming from studies in people with cholesterol concentrations below the criteria previously used by FSANZ to define 'normal' cholesterol concentrations. Consequently, there was no reason to repeat the analyses using the cut-off points previously used by FSANZ.

No analyses were carried out by sex because Professor Mensink advised that earlier work showed that this was not an important effect modifier. No analyses were performed by age because the inclusion criteria had specified that only adults would be included.

Studies of the effects of TFA on blood cholesterol concentrations are more recent than many of the studies of SFA, MUFA and PUFA. It is possible that older studies used food analysis methods that yielded total MUFA or PUFA intakes (which included *trans* MUFA or *trans* PUFA) rather than describing *cis*-MUFA or *cis*-PUFA intake. If so, then the effect of MUFA or PUFA would be muted in the older studies compare to more recent studies. To examine this, a subgroup analysis was pre-specified, dichotomising studies by publication before or after 1993, when studies reporting specific effects of TFA might have started to be reported.

As shown in Table 5, the effect of replacing classes of fatty acids with each other can be estimated from the sum of the effect of replacing each class of fat with carbohydrate. In the interest of succinctness, and because this shows the step-wise comparison in more detail, the subgroup analyses are only presented for the exchange of classes of fatty acids with carbohydrate. Subgroup analyses were not performed for the analysis examining individual fatty acids because LA is the predominant type of PUFA in the diet and so subgroup analyses would essentially replicate the results of its class.

3.2 Results

3.2.1 Search results

Professor Mensink had updated the literature search for his review in 2009 and then again in January 2014. In total, 74 studies with 2172 subjects and 177 diet strata were available for analysis of the total-C concentration outcome and 69 and 68 studies (2026 and 2017 subjects respectively) for LDL-C and HDL-C concentrations respectively. The search strategy and PRISMA diagram are shown in Mensink 2016 and Supporting Document 1. A description of the studies added in the update is given in Mensink (2016), Supporting Document 1 and in Appendix 1.

3.2.2 Quality assessment of individual studies

The studies are all trials which set out to test some aspect of the diet relating to variations in fatty acid intake, usually relating to effects on lipids. A few studies also stated hypotheses concerning effects on LDL-oxidation (Castro et al. 2000; Kratz et al. 2002; Binkoski et al. 2005), glucose metabolism (Lovejoy et al. 2002; Vega-Lopez et al. 2006; Berglund et al. 2007; Roussell et al. 2012) or haemostatic factors (Iggman et al. 2011).
A detailed description of the risk of bias for the papers is given in Mensink (2016) and summarised in Figure 1. Overall, there was little variability in the conduct of the included trials when considering the characteristics usually assessed to describe study quality. The characteristic missing from almost all studies was a description of whether allocation of subjects to the test order was done blind. The other criteria were achieved by most or all studies. Although most studies do not describe how the randomisation sequence is generated, this was rated as a low risk of bias in the cross-over studies (Mensink 2016). Furthermore, many studies were blind because the oils or sources of fatty acids were used as ingredients in meal preparation and virtually all food was provided to subjects.

The majority of meals were eaten under the supervision of trial staff, and the remainder packed for the subjects to take away. Researchers also required subjects to keep diaries of food eaten. Because fatty acid intake is calculated over the whole diet, it is not possible to give subjects a small number of intervention or control foods or supplements to achieve the difference in intake, which might be possible when many foods or food components are tested. In some studies, plasma or red cell membrane fatty acids of subjects (Lovejoy et al. 2002; Sabate et al. 2003; Lichtenstein et al. 2006; Rajaram et al. 2009) or urinary nitrogen (Poppitt et al. 2002) was tested to assess compliance. Subjects were weighed regularly and their total energy intake adjusted to ensure that body weight remained constant. Most of the new studies collected duplicate samples of the foods supplied to subjects and confirmed the fatty acid profile by chemical analysis.

Lack of comment as to whether laboratory personnel were blinded is not regarded as a source of possible risk of bias because auto-analysers have been available to test cholesterol fractions for many decades, although one study noted that both laboratory staff and the statistician performing data analysis were blinded (Judd et al. 2002). Thus the 74 studies are considered to have low risk of bias overall and are therefore high quality studies when compared to the excluded studies, which did not provide food to subjects.

All new studies except Kratz et al. (2002) used a cross-over design. A benefit of using a cross-over design is that many confounding factors related to subjects are controlled because the same people consume the various diets that are being compared. This can be an added benefit when samples sizes are small and randomisation can leave unbalanced baseline values in a parallel design. In most cases, the diets containing different amounts of fats were identical apart from the oil or fat used in their preparation, and so this further limits confounding by other nutrients or food components. An exception are the studies which tested nuts (Curb et al. 2000; Rajaram et al. 2001; Sabate et al. 2003; Rajaram et al. 2009) or meat (Roussell et al. 2012) which would contain other food components which might or might not affect blood cholesterol concentrations. These studies were funded by industry (see Section 3.3.2).

The minimum specified duration of 13 days is long enough to stabilise blood cholesterol concentrations (Brussaard et al. 1982; Mensink and Katan 1987) and allow observation of any effects. Therefore, provided that studies last for at least this duration, it is not necessary to have a wash-out period between diet phases. Some authors specifically mention testing their data for carry-over effects and that this was not observed in their results. This is consistent with the view that 13 days is a sufficient period for washout of any effect of the previous diet on blood cholesterol concentrations.

The studies in the existing review and those added in the update were published between 1970 to January 2014, a period of 43 years. During this time the types of food available have changed. However within each study, the diets were supplied by the investigators to all subjects. Therefore the results cannot be attributed to variations in the background diet. There was little loss of subjects to follow-up. In most studies, the number of subjects was small and so there could be baseline imbalances in important confounders even when randomisation is carried out well.

Few of the studies refer to having calculated study sample sizes and little detail is given even when this is mentioned. Because the within-person correlation in cholesterol concentrations is high at around 0.8 (Demonty et al. 2009; FSANZ 2015b), using a cross-over design means that a much smaller sample size is required to show that any specific difference in cholesterol concentrations is statistically significant, when compared to using a parallel design. Furthermore, many studies took two or three blood samples per study participant and averaged the results (see Appendix 1) and this would further reduce intra-individual variability and increase the power of the study.

It is difficult to compare the results shown in Appendix 1 directly to each other without doing a regression analysis owing to the wide range of intakes assessed, the differences in the proportions of fatty acids in the diets, and therefore the variation in the expected magnitude of effect. To detect differences in LDL-C of at least 0.3 mmol/L and 0.2 mmol/L as statistically significant, 19 and 50 subjects respectively would be required. This is assuming a two-sided alpha of 0.05, a power of 80% and a standard deviation of 0.8 mmol/L for LDL-C, and taking into account that a cross-over study requires only 10% of the sample size that a parallel study requires, if the correlation between two measurements of LDL-C is 0.8. This magnitude of difference would be predicted by a 10 %En exchange of carbohydrate for SFA or PUFA respectively (Table 5) or about a 5 %En exchange of SFA for PUFA. The sample sizes range from seven to 103 among the cross-over studies (Mensink 2016) and so many would have been adequately powered internally. Some studies were performed specifically to determine whether specific fatty acids had the same effect as each other, for example LA versus ALA (Zhao et al. 2004) or whether different sources of MUFA had the same effect as each other (Kris-Etherton et al. 1999). The sample size required to show no difference with a high degree of certainty is much larger than that required to find a statistically significant difference.

FSANZ concluded that the overall risk of bias in the body of evidence was low and that the 74 studies can be regarded as high quality studies.

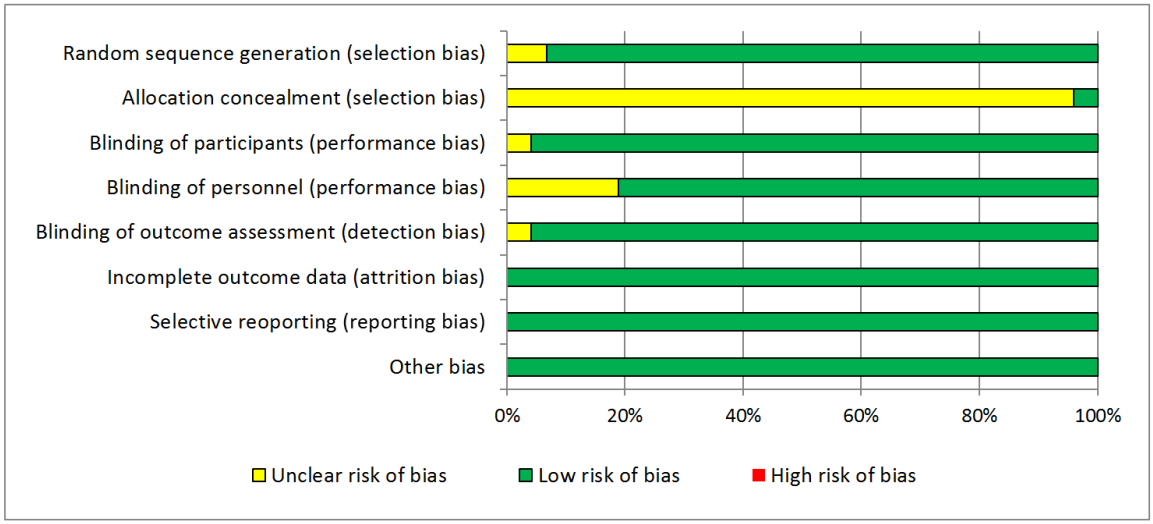


Figure 1. Risk of bias analysis of the 74 included studies (summarised from Annex 9, Mensink 2016).

3.3 Summary of evidence

Figure 2 shows a box plot of the mean fatty acid intake across all diets as the %En. The central box shows the interquartile range of mean intakes in the studies (that is the 25th to 75th centiles). It also shows the mean intake by adults in the most recent national nutrition surveys in Australia and New Zealand. As noted above, the percentages from the surveys include the contribution of glycerol whereas the percentages for the studies in the review do not. This difference is very small as glycerol contributes only about 4% to the total weight of a triglyceride. The small overestimation of the national intakes relative to the studies would be difficult to detect given the scale of Figure 2. It is evident that the mean intakes in both countries lie within the range tested in studies in the review, and in most cases, within the central 50% of intakes examined by the studies.

Mean intake of total fat in the 175 diets included in the review was 34.1 %En SFA (range 4.5 to 53.0 %En), with 9.8 %En (1.6 to 24.4 %En) from SFA, 13.6 %En (1.6 to 39.8 %En) from MUFA, and 8.4 %En (0.4 to 28.8 %En) from PUFA (Figure 2). Approximately two-thirds of the participants were men. The studies lasted between 13 to 90 days. Sixty-two trials reported the mean age of their participants, which varied between 21 and 72 years (mean 39 years). Mean BMI was reported for 56 studies and ranged between 20.3 and 28.6 kg/m^2 (mean 24.3 kg/m^2). For serum total-C (56 studies), mean pre-study concentrations ranged between 3.8 and 6.7 mmol/L (mean 5.1 mmol/L), for LDL-C concentration (48 studies) between 2.3 and 4.8 mmol/L (mean 3.4 mmol/L) and for HDL-C concentration (47 studies) between 0.9 and 1.8 mmol/L (mean 1.2 mmol/L). The number of diet data points included in the calculations varied from 177 (from 74 studies with 2172 participants) for changes in total-C concentration related to the classes of fatty acids to 87 (from 35 studies) for the analyses examining the effect of individual fatty acids on LDL-C and HDL-C concentrations (see Supporting Document 1).

3.3.1 Classes of fatty acids – exchange with saturated fatty acids or carbohydrate

Table 6 shows the results of two different multiple regression analyses. In the left-hand section (Columns 1, 2 and 3), the results for an isoenergetic exchange of SFA for MUFA, PUFA or carbohydrate is shown. In the right-hand section (Columns 4, 5 and 6) the results for an isoenergetic exchange of carbohydrate for SFA, MUFA or PUFA is shown. As would be expected, the effect of replacing SFA with an unsaturated fatty acid can be estimated by

considering the isoenergetic effect of each fatty acid class compared to carbohydrate. For example, the effect of replacing SFA with PUFA (Column 2) can be estimated from the effect of replacing SFA with carbohydrate (Column 3) and the effect of replacing carbohydrate with PUFA (Column 6).

Table 6 shows the effect of replacing SFA with either MUFA (Column 1) or PUFA (Column 2). There is a significant reduction in LDL-C concentration and the 95% confidence intervals are tight. The significant reduction in total-C concentration is slightly larger than the reduction in LDL-C concentration, presumably owing to the significant reduction in HDL-C concentration. However there is an overall net favourable change in the total-C/HDL-C ratio, indicating that the effect on LDL-C concentration is proportionally larger than the impact on HDL-C concentration. The regressions were weighted by the number of subjects for each data point; using either unweighted regression or weighting by the inverse of the variance did not alter the results importantly (R Mensink, Personal communication, 2014).

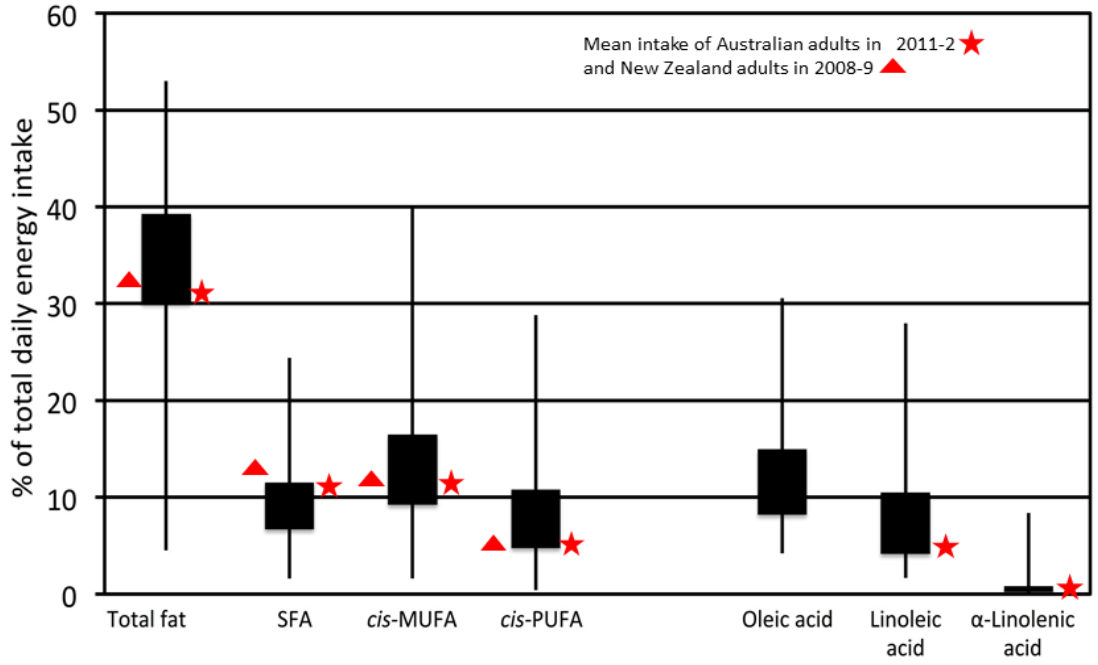


Figure 2. Box plot of the quantity of fats in the studies (expressed as a percent of energy intake) in the updated review (Source: Supporting Document 1, Figure 2) with the mean intake of the same by Australian and New Zealand adults reported in the most recent national nutrition surveys in each country (the solid rectangles indicate the 25th percentile and 75th percentile, and the vertical lines extend to the minimum and maximum intakes)

Column 3 of Table 6 shows the effect of reducing SFA intake with isoenergetic replacement by carbohydrate. This exchange reduces LDL-C, total-C concentrations and HDL-C concentration. However these effects are proportional to each other and so there is no effect on the total-C/HDL-C ratio. This contrasts with the effect of exchanging SFA for MUFA or PUFA which does reduce the total-C/HDL-C ratio.

For completeness and to allow comparison with the existing review (Table 6), Columns 5 and 6 show the effect of increasing MUFA and PUFA intake by isoenergetic reduction of carbohydrate intake. Column 5 shows that increasing MUFA intake by isoenergetic decrease in carbohydrate intake has little effect on the concentrations of total-C, LDL-C and HDL-C. Despite this, these small, individually non-significant effects yield a significant favourable improvement in the total-C/HDL-C ratio. By contrast, increasing the intake of PUFA while

decreasing carbohydrate intake leads to significant reductions in total-C and LDL-C concentrations and a significant increase in HDL-C concentration[9]. Comparing Columns 4-6 with Table 5 shows that the effects on cholesterol concentrations are very similar despite the addition of 25 new studies in the update.

[9] Column 4 shows the inverse of Column 3, i.e. the isoenergetic replacement of carbohydrate with SFA. Although it would be expected that Columns 3 and 4 would be the exact inverse of each other, there is a small numerical difference owing to the different expression of other variables in the models (see Supporting Document 1).

Table 6: Estimated multiple regression equations for the mean changes in serum lipids when one percent of energy in the diet from saturated fatty acids (SFA) is replaced isoenergetically by cis monounsaturated fatty acids (SFA → MUFA), cis polyunsaturated fatty acids (SFA → PUFA) or by carbohydrate (SFA → carb) and when one percent of energy in the diet from carbohydrates in the diet is replaced isoenergetically by saturated fatty acids (carb → SFA), by cis monounsaturated fatty acids (carb → MUFA) or by cis polyunsaturated fatty acids (carb → PUFA) (Source: Tables 1 and 2, Supporting Document 1)

	Change per percent of energy replaced			Change per percent of energy replaced			Number strata/studies /participants
	SFA → MUFA	SFA → PUFA	SFA → Carb	Carb → SFA	Carb → MUFA	Carb → PUFA	
	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	
ΔTotal cholesterol	-0.046	-0.064	-0.041	0.045	-0.004	-0.022	177/74/2172
95% CI	-0.051 to -0.040	-0.070 to -0.058	-0.047 to -0.036	0.039 to 0.051	-0.009 to 0.001	-0.027 to -0.016	
P-value	<0.001	<0.001	<0.001	<0.001	0.12	<0.001	
ΔLDL-cholesterol	-0.042	-0.055	-0.033	0.036	-0.009	-0.022	165/69/2069
95% CI	-0.047 to -0.037	-0.061 to -0.050	-0.039 to -0.027	0.030 to 0.043	-0.014 to -0.003	-0.028 to -0.015	
P-value	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	
ΔHDL-cholesterol	-0.002	-0.005	-0.010	0.011	0.008	0.006	163/68/2017
95% CI	-0.004 to 0.000	-0.006 to -0.003	-0.012 to -0.008	0.010 to 0.013	0.007 to 0.010	0.004 to 0.008	
P-value	0.014	<0.001	<0.001	<0.001	<0.001	<0.001	
ΔTotal to HDL-cholesterol	-0.027	-0.034	0.001	-0.002	-0.029	-0.036	159/66/1990
95% CI	-0.033 to -0.022	-0.040 to -0.028	-0.006 to 0.007	-0.009 to 0.005	-0.035 to -0.023	-0.043 to 0.029	
P-value	<0.001	<0.001	0.842	0.553	<0.001	<0.001	

The 95 percent confidence intervals (CI) refer to the regression coefficients on the preceding line.

3.3.2 Subgroup analyses for classes of fatty acids: exchange for carbohydrate

Type of carbohydrate: Five studies used liquid formula diets (with different types of fatty acids) in which all the carbohydrate was given as sugar. Figure 3 shows that excluding these studies from the analysis does not alter the results.

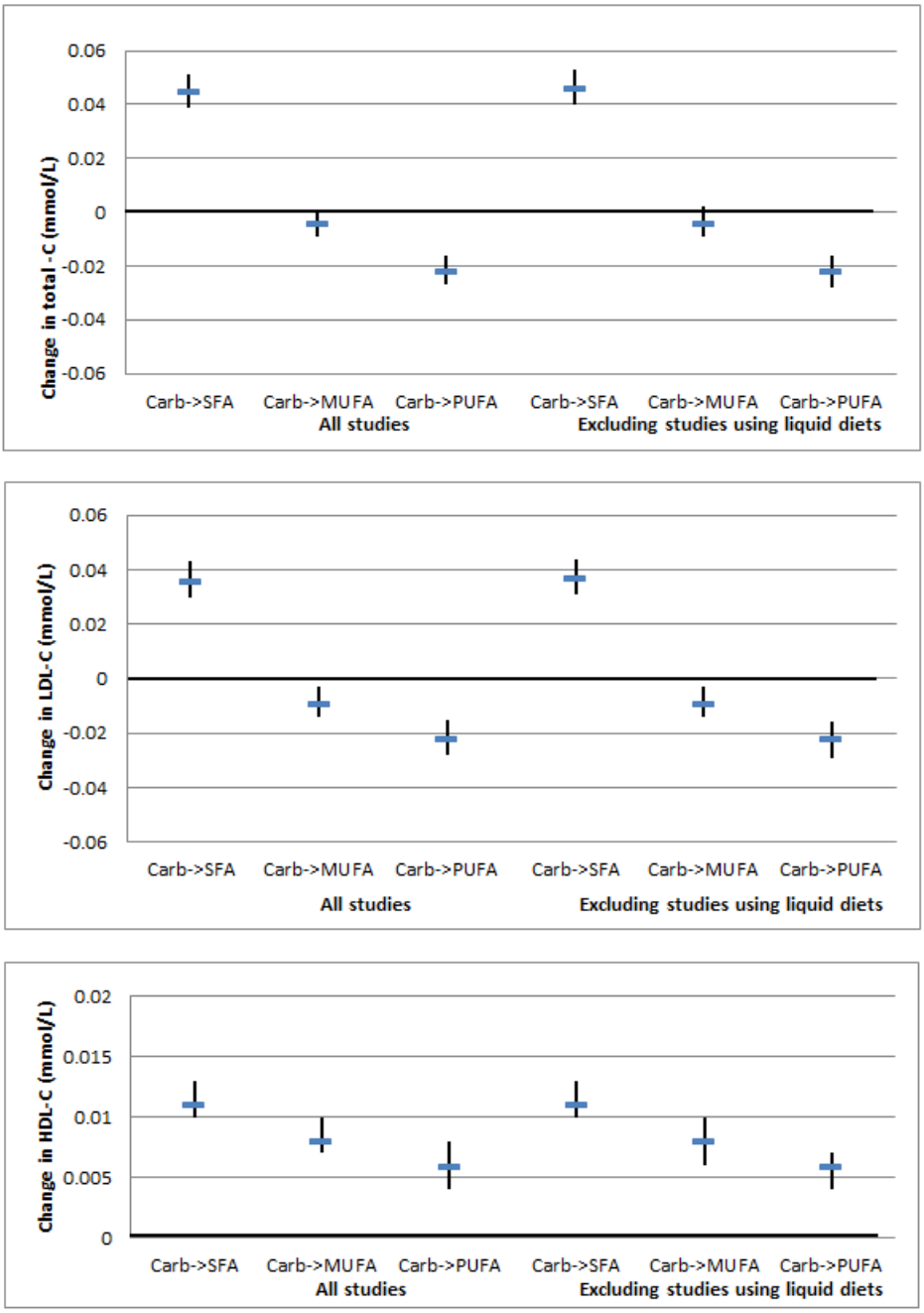


Figure 3. Change in total-C (top), LDL-C (middle) and HDL-C (bottom) concentrations (with 95% CI) when 1 percent energy from carbohydrate is replaced isoenergetically with three classes of fats for all studies and after excluding studies which provided liquid formula diets. The heavy line at 0 shows the point of equivalence with carbohydrate. (Source: Table 1 and Annex 5 of Supporting Document 1)

By baseline cholesterol concentration: Fifty-six of the 74 studies reported baseline cholesterol concentration of their subjects. The median concentrations when subjects consumed a standardized fat-free diet were 4.45 mmol/L for total-C, 2.89 mmol/L for LDL-C and 0.97 mmol/L for HDL-C concentrations. The same patterns were observed among those with baseline cholesterol concentrations above or below the medians (Figure 4). The effect of the Carb → SFA exchange was a little stronger in those with the higher baseline concentrations whereas the Carb → MUFA or PUFA exchanges were more similar.

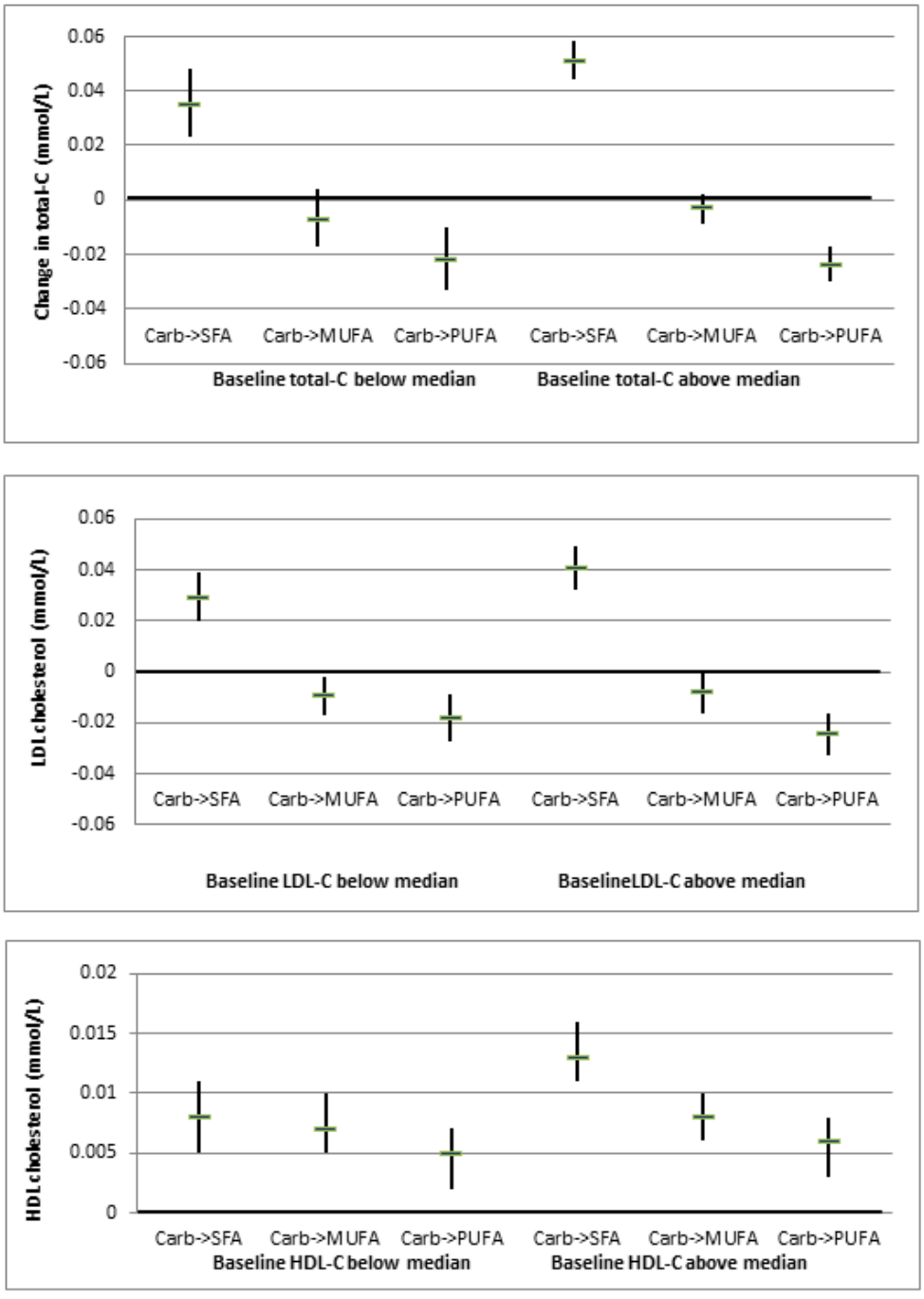


Figure 4. Change in total (top), LDL-C (middle) and HDL-C (bottom) concentrations (with 95% CI) when 1 percent energy from carbohydrate is replaced isoenergetically with three classes of fats, by baseline cholesterol concentration. The heavy line

at 0 shows the point of equivalence with carbohydrate. (Source: Annex 4 of Supporting Document 1)

By date of publication: The overall analysis was dichotomised by publication before 1993 or 1993 or later (Figure 5). Date of publication did not affect the results for either SFA or MUFA. However studies published from 1993 onwards show nearly a 50 percent larger effect on total-C and LDL-C concentrations for the exchange of carbohydrate for PUFA and a smaller effect on LDL-C for carbohydrate exchanged with MUFA.

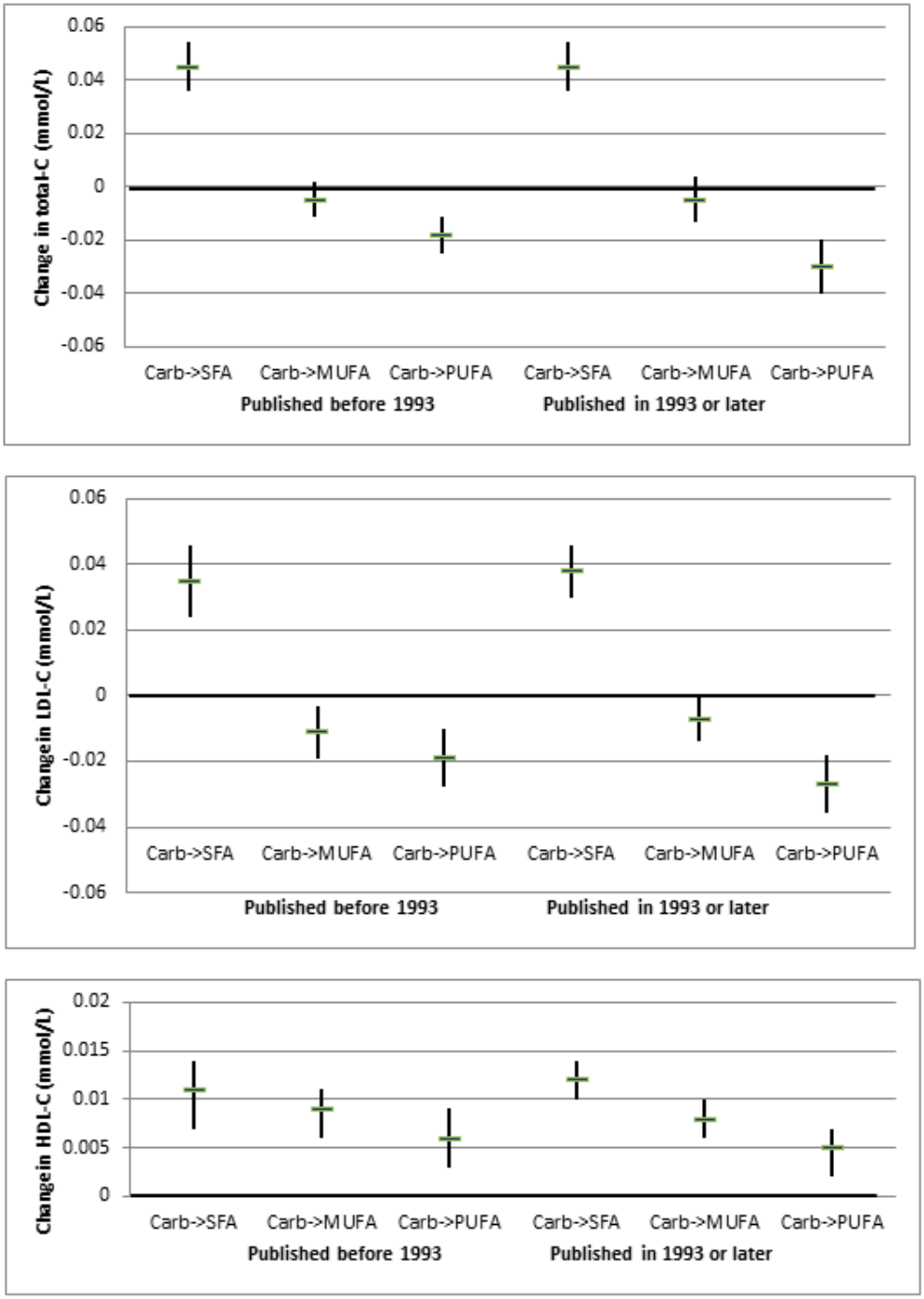
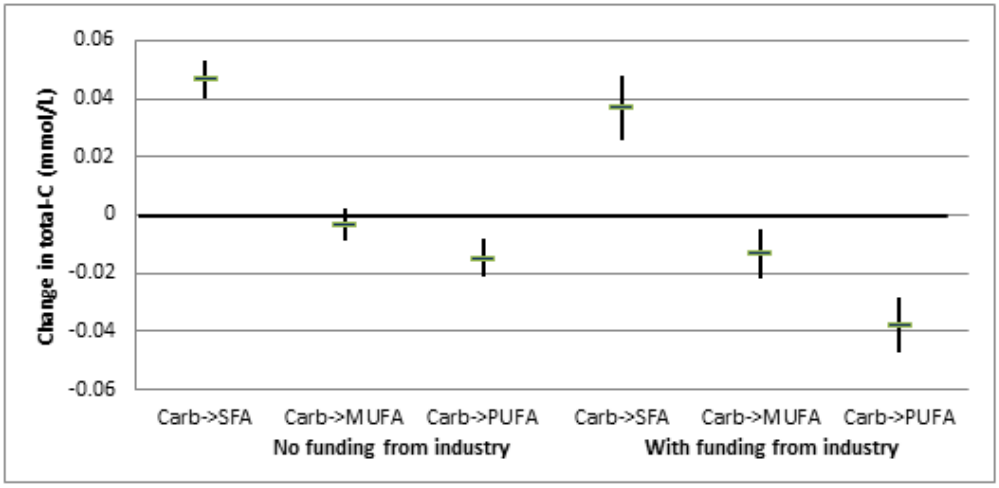
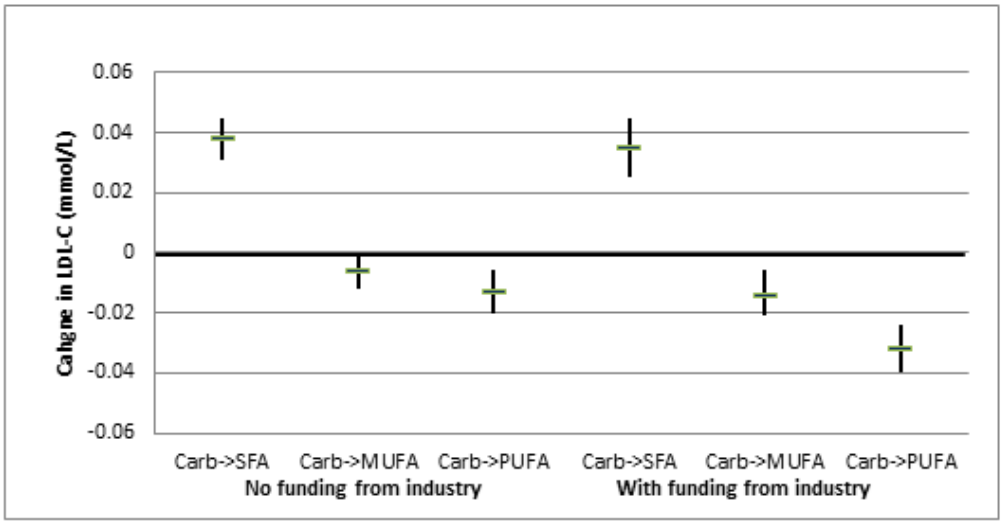


Figure 5. Change in total-C (top), LDL-C (middle) and HDL-C (bottom) concentrations (with 95% CI) when 1 percent energy from carbohydrate is replaced

isoenergetically with three classes of fats by publication date. The heavy line at 0 shows the point of equivalence with carbohydrate. (Source: Annex 6 of Supporting Document 1)

By industry funding: Studies were categorised according to whether they declared that they had received funding from at least one industry source (32 studies) or no industry funding (34 studies). Studies which received in-kind materials were classified as not-industry funded if they did not report receiving funding as well. Eight studies, which did not declare any sources, were excluded from this sub-analysis. The same general patterns were observed (Figure 6).





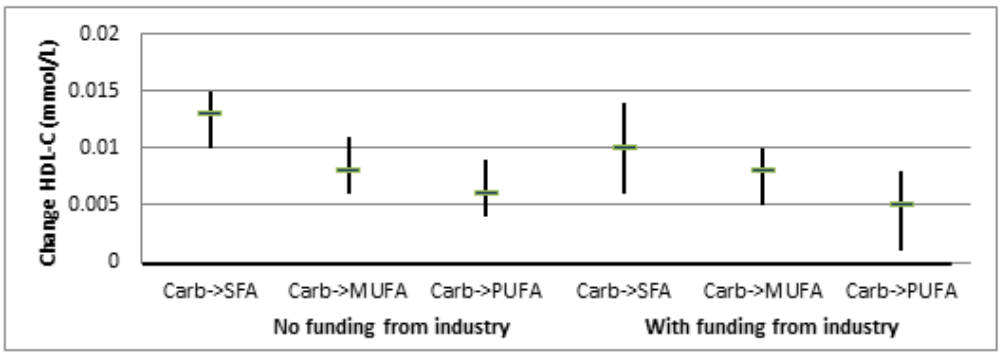


Figure 6 Change in total-C (top), LDL-C (middle) and HDL-C (bottom) concentrations (with 95% CI) when 1 percent energy from carbohydrate is replaced isoenergetically with three classes of fats, by source of funding. The heavy line at 0 shows the point of equivalence with carbohydrate. (Source: from Annex 7 of Supporting Document 1)

Notably, industry-funded studies reported a 3-fold larger effect of PUFA on reducing LDL-C (and therefore a larger effect on total-C) than did non-industry funded studies. This might, or might not, be related to inclusion of studies of whole foods - nuts and meat - rather than oils as sources of unsaturated fats, in the industry-funded group.

3.3.3 Individual PUFA: exchange with carbohydrate

Approximately half of the studies reported intakes of OA, LA and ALA. Figure 7 presents the effect of isoenergetic exchange of carbohydrate for SFA for all studies (177 strata as shown previously in Figure 3) and for the subset of 37 studies (91 strata) reporting information for OA, LA and ALA. As noted above, OA was included to adjust the analysis for any effects of MUFA. It is evident that the two results for Carb $\rightarrow$ SFA are very similar although the analysis with fewer strata has a wider 95% CI which would be expected due to the smaller sample size. Therefore, it can be concluded that, although nearly half the studies did not report the individual fatty acid data, the studies which provide this information are not likely to be a biased subset of the total.

The majority of PUFA in the Western diet is LA. Therefore, as would be expected the isoenergetic exchange of carbohydrate for LA is similar to that observed for PUFA as a class. The effect of Carb $\rightarrow$ ALA appears to be stronger than Carb $\rightarrow$ LA, but the 95% CI is much wider and there is considerable overlap of the two 95% CIs. As shown in Figure 2, the range of LA intakes tested is much wider than the range of ALA intakes tested. Therefore, it can be concluded that increased ALA intake lowers total-C and LDL-C when exchanged for carbohydrate, but it is less certain whether the effect of ALA is importantly different from the effect of LA. The model also contained Carb $\rightarrow$ OA to adjust for the effects of MUFA and this is also shown in Figure 7 for completeness.

3.3.4 Publication bias

The results for the classes of fatty acids show little variation over time (Table 5) and are consistent with pre-1970 estimates for total-C concentration, such as that described in the Keys equation (Keys et al. 1965). In addition, examination of the residuals for SFA from the regression for LDL-C shows that a linear model is an excellent fit to the data (Annex 5, Mensink 2016). The residuals are homoscedastic, i.e. there is no variation in the variability of the results among studies that relates to the amount of SFA (Annex 4, Mensink 2016).

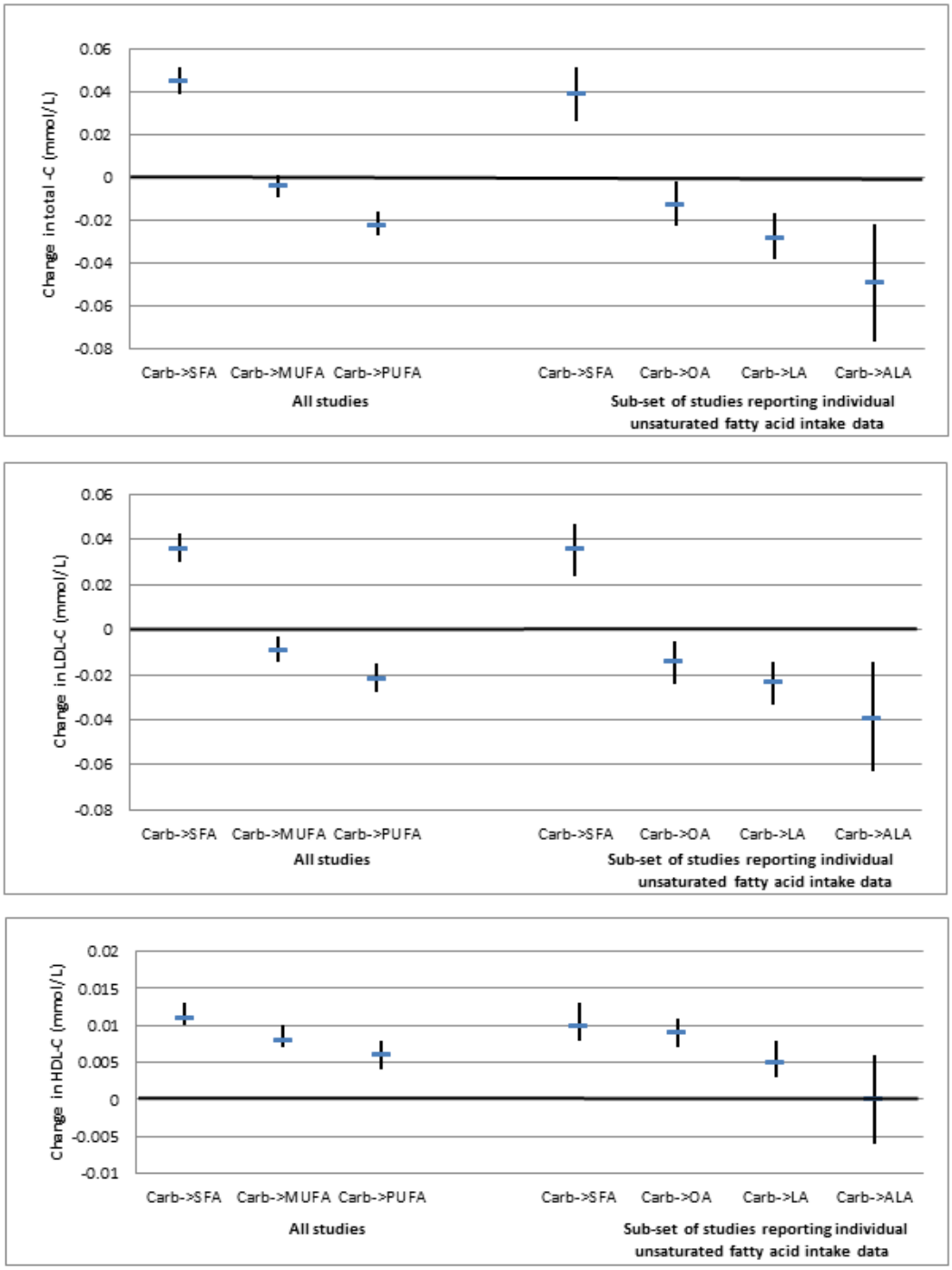


Figure 7. Change in total-C (top), LDL-C (middle) and HDL-C (bottom) concentrations (with 95% CI) when 1 percent energy from carbohydrate is replaced isoenergetically with three classes of fats (left-hand set) or by saturated fatty acid or three individual fatty acids in the subset of studies with this information (right-hand set). (The heavy line at 0 shows the point of equivalence with carbohydrate. Source: Table 3 of Supporting Document 1)

3.4 Summary of results

Owing to the multiple relationships and analyses covered in this review, and the complexity in describing the nature of the macronutrient exchange, the direction determined from the analysis is summarised qualitatively in Table 7. All the analyses described below can also be described in the inverse, for example ‘decreasing SFA intake with isoenergetic replacement by PUFA decreases LDL-C concentration’ could be re-expressed as ‘increasing SFA intake with isoenergetic reduction in PUFA increases LDL-C concentration’ or ‘increasing PUFA intake with isoenergetic reduction by SFA decreases LDL-C concentration’.

As noted above, PUFA refers to the 18 carbon atom PUFA because the review excluded studies that focused on increasing the intake of the long chain omega-3 fatty acids EPA and DHA. Although studies which focused on increasing intakes of arachidonic acid were not excluded, no such studies were found and so no conclusions can be drawn about whether or not the results observed for PUFA would apply to arachidonic acid.

Table 7: Qualitative summary of the results of the analysis

Column 1	Column 2	Direction of change in	
		Total-C	LDL-C
When SFA intake decreases with isoenergetic replacement by the component in Column 2	carbohydrate	decrease	decrease
	MUFA	decrease	decrease
	PUFA	decrease	decrease
When carbohydrate intake decreases with isoenergetic replacement by the fatty acid in Column 2	LA	decrease	decrease
	ALA	decrease	decrease

4 Weight of evidence

A large number of trials published since 1970 reporting results in adult participants were available for analysis. The addition of 25 studies to the existing review, did not change the overall conclusions drawn from the existing review. The studies were conducted in adults, with both normal and elevated cholesterol concentrations. The body of evidence was regarded as high quality owing to the inclusion criterion that most or all of the food had to be supplied to subjects by the investigators and this eliminated errors related to the need for subjects to understand and implement dietary instructions. There was little variation in results by dietary type, baseline cholesterol status, funding source or period that the study was conducted.

4.1 Assessment of body of evidence

4.1.1 Consistency of relationship

In the dataset, there were 74 studies published over more than 40 years by a range of research groups across a number of countries. These studies tested alterations to base diets that ranged from ‘average’ Western with 35-40 %En from total fat to more restricted Step II

diets which specify a maximum of 25 %En from total fat and 7 %En from SFA (Goodman et al. 1988). Owing to the time span of the data, the oils tested ranged from the original unsaturated oils such as sunflower and safflower to the modern oils such as canola, flaxseed and high oleic acid sunflower oil. The updated review (Supporting Document 1; Mensink 2016) does not alter the conclusions of the existing review in any important way. It adds some detail regarding individual fatty acids, which are consistent with their being the major components of MUFA and PUFA classes.

The effect of replacing carbohydrate with SFA on total-C is approximately double that of replacing with PUFA, but in the opposite direction. This is essentially the same result as the existing review (Mensink et al. 2003). Previously, in 1965, Keys also described a two-fold and opposite effect of SFA and PUFA on total-C cholesterol (Keys et al. 1965). Sub-analyses by period of publication, source of funding or baseline cholesterol concentrations yielded broadly similar results. Regression diagnostics showed that the model was well behaved and that there were no influential outliers.

4.1.2 Causality

Randomised controlled trials are a strong design for inferring causality. Most of the studies used a cross-over design which further reduces possible variations between groups related to background characteristics. The analysis involving an isoenergetic exchange means that the results are not attributable to concurrent changes in body weight.

The inclusion criteria used in the review restricted the analysis to studies in which the authors had good control over what the subjects ate and so are not affected by low adherence or inaccurate reporting of dietary intake and therefore the studies are considered to be high quality studies. The studies measured total-C and HDL-C directly. LDL-C concentrations were generally calculated using the Friedewald equation, which is the method in common clinical use and so the degree of certainty was not down rated for indirectness in the outcome. The overall sample size is large and confidence intervals around the regression coefficient estimates are narrow and so there was no down rating for imprecision. Consistency in the results from studies in a range of locations over more than four decades indicates that the results are not due to publication bias. The majority of studies were conducted in people who would be regarded as having normal cholesterol concentrations.

FSANZ concludes that there is a high degree of certainty that exchanging SFA for carbohydrate, MUFA, or PUFA decreases total-C and LDL-C concentrations and that this is causal. Similarly, FSANZ concludes that there is a high degree of certainty that replacing carbohydrate with LA or ALA decreases total-C and LDL-C and that this is causal. There was no variation by baseline cholesterol concentration and so the above conclusions apply to people with normal cholesterol concentrations.

4.1.3 Plausibility

Fatty acids are ligands of several regulatory pathways which can play a role in determining plasma cholesterol levels. Mechanistic studies have reported that saturated fatty acids increase plasma LDL-C by increasing LDL formation and by decreasing LDL turnover. The lowering of plasma LDL-C observed with PUFA is likely due to redistribution of cholesterol between plasma and tissue pools and upregulation of the LDL receptor. Although unsaturated fatty acids have been shown to increase cholesterol synthesis, they also increase hepatic LDL receptor number and LDL turnover. Possible mechanisms by which omega-6 PUFAs decrease plasma cholesterol include upregulation of the LDL receptor and increased metabolising enzyme (CYP7) activity, whereas omega-3 PUFAs decrease plasma triglycerides by decreasing lipogenesis and secretion of very low density lipoprotein,

increasing lipoprotein lipase activity, and increasing reverse cholesterol transport (Fernandez and West 2005).

4.2 Applicability to Australia and New Zealand

4.2.1 Intake required for effect

Figure 2 shows the range of baseline fatty acid intakes in the study populations from which the effect of changing fatty acid intakes on cholesterol concentrations was calculated. The mean intakes of adults in Australia and New Zealand are in the central part of this distribution. Therefore FSANZ concludes that the effects of a 1 %En exchange in intake of the fatty acids found in regression analyses applies at the current population mean intake. FSANZ further notes that the regression was clearly linear (Mensink 2016) and consequently there is no minimum exchange at which the relationship operates.

Standard 1.2.8 – Nutrition information requirements stipulate a reference value of 8700 kJ for percent daily intake declarations in the nutrition information panel[10]. For this level of energy intake, 1% is supplied by 2.35 g fat.

4.2.2 Relevant population

Although the number of participants within studies was small, across the studies the participants had a wide range of ages, were normal weight or overweight, with serum cholesterol concentrations in the normal to slightly elevated range, and were generally healthy. Typical exclusions were people with kidney, liver, thyroid and similar types of conditions, and those taking medications that might alter cholesterol concentrations. Some studies included women taking oral contraceptives or people who smoked, whereas other studies excluded these individuals.

One study was conducted in New Zealand (Poppitt et al. 2002), one in Malaysia and two in Israel. Approximately half the studies were conducted in the US and the remainder in Canada or Europe. The first of the included studies was conducted in 1970 and the most recent in 2012 and so all studies would have been conducted in study groups with background diets that broadly reflect the range of foods available in Australia and New Zealand over that period, despite the diversification of the diet over the same time. There is no important difference in the results for studies conducted before or from 1993 and updating the existing review did not alter its conclusions. Furthermore, more than half of all studies were conducted in people who have normal (≤5.5 mmol/L total-C) total-C concentrations and the effect was seen in those with lower and higher baseline total-C concentrations. Therefore the relationships are applicable to normocholesterolaemic generally healthy Australian and New Zealand adults.

As the review was restricted to adult populations, the evidence assessed does not provide data to substantiate the relationship between any of the fatty acids examined and blood total-C or LDL-C concentrations in children.

4.2.3 Extrapolation from supplements

There is no need for extrapolation because the majority of the studies used mixed foods to vary the fatty acid intake. Excluding five studies testing liquid formula diets from the analysis did not alter the overall results.

[10] https://www.legislation.gov.au/Series/F2015L00395

4.2.4 Adverse effects

The effect on HDL-C concentration was examined because adverse effects on this parameter would be relevant when considering the evidence for a claim referring to reducing total-C or LDL-C concentrations. The total-C/HDL-C ratio was used to compare the effects on HDL-C relative to other effects. Even when HDL-C was reduced, there was either no change in total/HDL-C ratio or a favourable improvement in the ratio. Therefore the reduction in HDL-C seen was not considered to be an adverse effect.

5 Conclusion

The included studies are regarded as a high quality body of evidence. The results did not vary by baseline blood cholesterol concentration and so apply to people with normal cholesterol concentrations. The results for PUFA are based on studies which altered the amounts of the 18-carbon PUFAs, i.e. LA and ALA. The data do not allow conclusions to be drawn regarding the effects of other PUFA such as arachidonic acid, EPA or DHA on blood cholesterol concentrations. In addition, the studies of SFA did not examine the effects of altering the intake of short and medium chain saturated fatty acids.

FSANZ considers that the analyses used to assess the relationships need to consider the effect of substituting one macronutrient for another on an isoenergetic basis so that the confounding effects of changes in body weight are excluded. A different type of data that also allowed variation in energy intake (or therefore body weight) would not allow unambiguous attribution of effects on cholesterol concentrations to the change type of fat in the diet alone, and therefore substantiation of the relationships. Therefore the approach to substantiation has considered the effect of substituting one macronutrient for another on an isoenergetic basis, without concomitant changes in body weight, on the concentration of total-C and LDL-C.

Using the GRADE framework, it was concluded that there is a 'High' degree of certainty for all analyses investigated. The relationships are substantiated in people with normal cholesterol concentrations and for the current level of fatty acid intakes in Australia and New Zealand.

6 Acknowledgement

FSANZ gratefully acknowledges the updated review and analyses provided by Professor Mensink.

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Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM (2004) Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr 134:2991–2997

Appendix 1: Characteristics of the studies included in the analysis – *supplementary information for the 25 new studies shown in Annex 3 of Supporting Document 1 and one older study meeting revised inclusion criteria)*

Reference (First author, year of publication)	**Objectives stated by author[@]**	**Participants & sample size**	**Interventions[@,#]**	**Methods of outcome measurement**	**Confounders**	**Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)***
Hunter 2000	To assess the effect of isoenergetic diets differing in content of stearic acid, oleic acid, or linoleic acid on lipids, haemostatic variables, platelet function	Non-Smoking young men with BMI between 20-28 BMI: 24.7 No sample size calculations given	Residential study; all food provided by researchers; onion, garlic, certain spices, alcohol excluded; test oils added to cooked dishes such as potato, pasta etc. Test diet composition determined by chemical analysis	Total-C: kit from Sigma LDL-C: calculated by difference from total-C and HDL-C	Controlled by cross-over design Weight assessed alternate days Adverse/other effects: CRP, TG, various clotting factors,	Total-C, LDL-C Diet 1: 3.9, 3.1 Diet 2: 3.7, 2.6 P: not reported No carry-over effects detected
Judd 2002	To assess the effect of equicaloric replacement of carbohydrate by stearic acid, TFA, OA and SFA	Men within 85-120% of ideal body weight; TG<3.39 mmol/L; HDL-C>0.65; smoker s not excluded No sample size calculations given	All food provided by researchers; black tea, black coffee , diet soft drink allowed in unlimited amounts Base diet with different fats used to prepare foods such as margarine, salad dressings etc to obtain desired fatty acid profiles Test diet composition determined by chemical analysis	Total-C: enzymatically with commercial kits by Sigma LDL-C: Friedewald equation 2 blood samples averaged Laboratory analysts were blind to diet	Controlled by cross-over design Weight assessed 5xweek Adverse/other effects: TG, HDL, lipoproteins	Total-C, LDL-C Diet 1: 4.7, 3.1 Diet 2: 4.6, 3.0 Diet 3: 4.8, 3.1 P: <0.01 for Diet 2 vs 3 on pairwise tests No significant carry-over effects; no interaction between diet and period Statistician was blind to allocation for preliminary analysis

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Vega-Lopez 2006	To assess the effect of consuming palm oil versus partially hydrogenated soybean, canola oil or soybean oil on lipids and indicators of glucose homeostasis	Non-smoking adults aged over 50 years, with LDL>3.36 BMI: 26.0 No sample size calculations given	All food provided by researchers; same food in all phases, prepared with different oils giving 20% energy Test diet composition determined by chemical analysis	Total-C: CCX, Spectrum; Incstar, Stillwater, MN LDL-C: calculated from total-C, HDL-C and VLDC-C 3 blood samples averaged	Controlled by cross-over design Energy intake adjusted to maintain stable body weight Adverse/other effects: TG, lipoproteins, indicators of glucose homeostasis and HDL metabolism	Total-C, LDL-C Diet 1: 6.2, 4.3 Diet 2:5.4, 3.6 P<0.05
Lichtenstein 1999	To assess the effect of various commercially available margarines & a vegetable shortening with a wide range of TFA to butter on lipids	Non-smoking adults aged over 50 years, with LDL>3.36 BMI: 27.4 No sample size calculations given	All food provided by researcher; additional water and non-energy beverages allowed; Step 2 diet with soybean oil or semiliquid margarine used to prepare foods Test diet composition determined by chemical analysis	Total-C: with a biochromatic analyzer (model CCX, Spectrum, Incstar, Stillwater, Minn.) with enzymatic reagents LDL-C: not stated 3 blood samples averaged	Controlled by cross-over design Weight assessed 4xweek Adverse/other effects: HDL, TG, lipoproteins,	Total-C, LDL-C Diet 1: 5.8, 4.0 Diet 2: 5.9,4.0 P: NS

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Lovejoy 2002	To assess the effect of diets enriched in SFA, TFA and MUFA on measures of insulin sensitivity	Non-smoking non-obese adults with LDL and TG between 5th-95th centile BMI: 23.5 A priori power analysis: 22 subjects would provide >85% power to detect a difference in *S*I of 0.45 units between diets, which would be clinically meaningful in subjects with average insulin sensitivity.	All food provided by researchers; alcohol prohibited; specially formulated blends developed to achieve isolated exchange for oleic acid for SFA used in baking etc Test diet composition determined by chemical analysis	Total-C: Beckman Synchron CX7 LDL-C: Friedewald *(S*I reflects the effect of an incremental change in plasma insulin to increase fractional glucose clearance independent of glycaemia.)	Controlled by cross-over design Energy adjusted to maintain stable body weight if weight varied more than 2 kg/wk Adverse/other effects: HDL, TG, glucose, insulin sensitivity	Total-C, LDL-C Diet 1: 3.8, 2.2 Diet 2: 3.9, 2.2 P: NS
Berglund 2007	To assess the effect of the Step 1 diet, a MUFA-containing diet and an average American diet on overall risk factor profile	Healthy individuals with one or more of the following: low HDL-cholesterol, high triacylglycerol, or high insulin concentrations BMI: 27.6 No sample size calculations given	All food provided by researchers except Saturday evening meal (although a Step 1-type meal was advised). Test diet composition determined by chemical analysis	Total-C: enzymatic assay (not further described) LDL-C: Friedewald 3 blood samples averaged	Controlled by cross-over design Fibre 7.5 g higher on CHO diet Weight assessed 2/week Adverse/other effects: HDL, TG, lipoproteins uric acid, glucose, insulin	Total-C, LDL-C Diet 1: 5.2, 3.3 Diet 2: 4.9, 3.1 Diet 3: 4.9, 3.1 P<0.01 for Diets 2 and 3 vs Diet 1 on pairwise tests Statistical analysis adjusted for seasonal variation

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Binkoski 2005	To assess the effect of a Step 1 diet with additional higher-PUFA sunflower oil or olive oil and an average American diet on susceptibility of LDL-C to oxidation	Men and women with moderate hypercholesterolemia LDL-C: 5.69 BMI: 26.1 No sample size calculations given	All food provided by researchers, additional non-energy drinks and seasonings permitted. Average American diet vs Step 1 diet modified to replace fats with one of the test oils which were used in preparing foods. Test diet composition determined by chemical analysis	Total-C: Infinity Cholesterol Reagent (procedure 401) from Sigma Diagnostics LDL-C: Friedewald 2 blood samples averaged	Controlled by cross-over design Weight assessed 5/week Adverse/other effects: HDL, TG, lipoproteins, LDL-C oxidation measures; plasma tocopherol	Total-C, LDL-C Diet 1: 5.8, 3.8 Diet 2: 5.7, 3.7 Diet 3: 5.5, 3.5 P<0.05 for pairwise tests of Diet 3 vs the other two diets
Castro 2000	To assess the effect of adding MUFA from either virgin olive oil or refined oil or sunflower oil to a Step 1 diet on the susceptibility of LDL to oxidation	Spanish medical students BMI: 24.3 No sample size calculations given	All food provided by researchers. 10% En from carbohydrate in the Step I diet replaced by oil used in food preparation Test diet composition determined by chemical analysis	Total-C: enzymatic assay (Boehringer Mannheim) LDL-C: enzymatic assay (Boehringer Mannheim) Lab supervisors were blind to diet assignment	Controlled by cross-over design Weight assessed 2/week Adverse/other effects: HDL, TG, lipoproteins, LDL-C oxidation measures	Total-C, LDL-C Diet 1: 4.3, 2.6 Diet 2: 4.0, 2.4 P<0.05
Kris-Etherton 1999	To assess the effect of a Step II diet to modified Step II diets with 3 difference sources of MUFA (olive oil, peanut oil, or peanuts and peanut butter)	Normocholesterolaemic men and women LDL-C: 3.05 BMI: 20-27 Sample size based on their previous studies and expected response (not further described).	All foods provided by researchers; additional non-energy containing beverages permitted. 9 %En from carbohydrate in Step II diet replaced with fatty acids from test foods Test diet composition based on chemical analysis of the food	Total-C: enzymatic assay, not further described LDL-C: Friedewald 2 blood samples averaged	Controlled by cross-over design Subjects maintained weight to within 1 kg Adverse/other effects: HDL, TG, lipoproteins	Total-C, LDL-C Diet 1: 4.9, 3.0 Diet 2: 4.8, 3.0 Diet 3: 4.9, 3.1 Diet 4: 4.8, 3.0 P: possible typographical error for total-C; NS for LDL-C

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Nielsen 2002	To compare the effects of two oleic acid rich diets (from olive oil or rapeseed oil) to sunflower-seed oil lipids	Non-smokers Total-C: 4.71 BMI: 22.9 Power calculations: 18 subjects would have power 0·85, at alpha=0.05 to detect a difference of 0·2 mmol/l in total -C.	All foods provided by researchers; additional water, and in small amounts, plain coffee, and tea allowed Base diet of 30% energy with 50 g/10 MJ of rapeseed oil, extra virgin olive oil or sunflower seed oil incorporated into bread, cakes, etc. Test diet composition based on chemical analysis of the food	Total-C: Boehringer-Mannheim B-M CHODPAP 236-691 LDL-C: calculated as the difference between HDL-C concentration and the cholesterol concentration in the LDL+HDL fraction	Controlled by cross-over design Weight assessed every second day; energy intake adjusted if weight varied more than 1 kg Adverse/other effects: HDL, TG, tocopherol, carotenes	Total-C, LDL-C Diet 1: 3.5, 2.2 Diet 2: 4.1, 2.7 Diet 3: 3.6, 2.3 P<0.001 by ANOVA No period or carry-over effects were detected
Poppitt 2002	To compare the effect of a natural butter-fat, modified to replace a proportion of saturates with MUFAs and PUFAs to regular butter could improve risk factors including lipids	Men with normal weight Weight: 69 kg No sample size calculations given	Residential study providing all food and beverages Pasture-fed cows given encapsulated unsaturated fat to alter butter fat composition. Test and regular butter with similar colour and hardness, used in meals and snacks, food preparation for subjects. Test diet composition based on chemical analysis of the food	Total-C: method not stated LDL-C: method not stated 2 blood samples averaged	Controlled by cross-over design Weighed daily; weight remained within 2 kg. Adverse/other effects: HDL, TG, glucose, insulin, lipoproteins, clotting factors	Total-C, LDL-C Diet 1: 4.3, 2.9 Diet 2: 4.2, 2.7 P<0.05 (total-C); <0.01 (LDL-C) by ANOVA

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Rajaram 2001	To compare the effect of replacing 20% energy in Step 1 diet with 72 g pecan nuts versus Step 1 diet on blood lipids and lipoproteins	Non-smokers; normocholesterolaemic, BMI: <30 Sample size: to detect mean differences of 0.26 mmol/L for total-C and LDL-C, 22 participants need to complete the study (alpha: 0.05; power .0.9)	Food provided by researchers; only additional water allowed. 72 g pecans replaced portion of entire diet by reducing serve size of all items on menu. Pecans served plain, in salads, gravies, shakes and as toppings. Test diet composition based on chemical analysis of the food	Total-C: 550 Express Chemistry Analyzer LDL-C: 550 Express Chemistry Analyzer 2 blood samples averaged Laboratory personnel blind to diet group.	Controlled by cross-over design Frequent weight monitoring; energy intake altered as needed; pecan phase weight was 0.4 kg lower Adverse/other effects: HDL, TG, lipoproteins,	Total-C, LDL-C Diet 1: 4.8, 3.1 Diet 2: 4.5, 2.7 P<0.001 No carry-over effects were detected Weight loss adjustment
Sanders 2003	To compare the effect diets containing TFA, carbohydrate or OA on clotting factors	Non-smokers, normo-lipidaemic young men LDL: 2.75 BMI: 24.2 Post hoc power calculation: 80% power to detect a 5% difference in FVIIc and fibrinogen between pairs of diets (P<0·05).	Residential study providing all food and beverages; permitted additional beverages: black tea, white tea, diet drinks ad lib, up to 4 cups coffee/day, up to 2 standard alcoholic drinks/day except 3 days before blood sampling Based on Step 1 diet, 10% energy difference due to carbohydrate (bread, sucrose) or oleic acid. Test diet composition based on chemical analysis of the food	Total-C: enzymatic and Immune-turbidometric assays, (reference given) LDL-C: as above 2 blood samples averaged	Controlled by cross-over design Matched vitamin E content of the diets. Adverse/other effects: HDL, TG, clotting factors	Total-C, LDL-C Diet 1: 4.2, 2.5 Diet 2: 4.3, 2.5 P: NS No carry-over effects were detected

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Wagner 2001	To compare the effects of a diet containing a MUFA-rich oil mixture of olive oil/sunflower oil versus PUFA-rich corn oil diet on plasma and lipoprotein lipid concentrations	Non-smoking young men BMI: 20.3 No sample size calculations given	Food provided by researchers supplied 90% energy; remainder was free choice of alpha-tocopherol-free foods. Base diet designed to meet dietary recommendations; test oils used in main dishes, pastries etc. Test diet composition calculated	Total-C: CHOD-PAP (Boehringer Manheim) LDL-C: not stated	Controlled by cross-over design Weight change <0.6 kg Adverse/other effects: HDL, TG, tocopherol	Time 1 Total-C, LDL-C Diet 1: 4.3, 3.1 Diet 2: 4.5, 3.9 Time 2 Diet 1: 3.9, 3.3 Diet 2: 4.6, 3.3 P<0.05 for LDL-C at Time 1 and total-C at Time 2, analysed as 2 parallel studies
Kratz 2002	To compare the effect of containing different amounts of MUFA, omega-6 PUFA and omega-3 PUFA on LDL oxidation and oxidisability	Non-smoking college students LDL-C: 2.98 BMI: 23.1 No sample size calculations given	Food provided by researchers supplied 90% energy, free choice of beverages or fruit which contained trace amounts of protein, fat, cholesterol from a list. Specific mention of matching vitamin E content of the diets Sunflower, olive or rapeseed oil used in food preparation. Test diet composition calculated	Total-C: enzymatic assays (reference given) LDL-C: Friedewald	Parallel design Weight assessed 2/week Matched vitamin E content of the diets. Adverse/other effects: HDL, LDL-oxidation	Total-C, LDL-C Diet 1: 3.9, 2.1 Diet 2: 4.2, 2.3 Diet 3: 4.1, 2.3 P: to compare diets not reported
Lichtenstein 2006	To compare the effect to of novel soybean oils with modified fatty acid profiles, relative to soybean and partially hydrogenated soybean oils, on risk factors	Non-smoking middle-aged and older moderately hypercholesteraemic adults LDL-C: 3.84 BMI: 26.7 No sample size calculations given	All food provided by researchers; unlimited additional water, diet beverages permitted Fat content of diet varied by using low-SFA soybean oil, high oleic soybean oil of low ALA soybean oil in food preparation Test diet composition based on chemical analysis of the food	Total-C: Roche Diagnostics reagents LDL-C: Roche Diagnostics reagents 3 blood samples averaged Laboratory staff blind to diet order	Controlled by cross-over design Weight assessed 3/week Adverse/other effects: HDL, TG, lipoproteins, CRP	Total-C, LDL-C Diet 1: 5.7, 3.7 Diet 2: 5.6, 3.5 Diet 3: 5.7, 3.7 Diet 4: 5.7, 3.7 P: NS

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Motard-Belanger 2008	To compare the effect of ruminant versus industrial TFA on total-C and LDL-CA on plasma LDL-cholesterol and other risk factors.	Non-smoking young men, 18-30 No sample size calculations given	Cows given safflower oil to alter composition butter derived from their milk. This butter or oils used to vary TFA content while limiting variation in SFA content of diet. All food provided by researchers; unlimited additional water, caffeine-free diet beverages permitted; tea and coffee restricted to 500 mL/day. Fatty acid profile of test fats determined by chromatography; diet composition calculated using food tables.	Total-C: not specified (references given) LDL-C: not specified (references given)	Controlled by cross-over design Weight assessed 5/week; energy intake altered to keep weight constant Adverse/other effects: HDL, TG, CRP, lipoprotein	Total-C, LDL-C Diet 1: 4.8, 3.3 Diet 2: 4.7, 3.2 P: NS Carry-over effects were tested for
Rajaram 2009	To compare the effect of walnuts or fatty fish versus a healthy diet on serum lipids	Non-smoking /mildly hyperlididaemic adults LDL-C: 3.53 BMI: 24.8 Sample size : 18 subjects needed in a crossover design to detect a mean difference in serum LDL-C of 0.26 mmol/L, alpha=0.05, power>0.9	All food provided by researchers Control diet consistent with dietary guidelines but without omega-3 rich food (nuts or seafood) versus 42.5 g walnuts substituted for meat/dairy food 6 days/week. Nuts eaten alone or in salads etc. Test diet composition calculated using food tables.	Total-C: enzymatic colorimetric assays with the Bayer 550 Express Chemistry Analyser LDL-C: as above 2 blood samples averaged	Controlled by cross-over design Weight assessed 2/week Adverse/other effects: HDL, TG, lipoprotein	Total-C, LDL-C Diet 1: 5.1, 3.1 Diet 2: 4.9, 2.8 P<0.05

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Gillingham 2011	To compare the effect of high oleic canola oil or a flaxseed/high-oleic rapeseed oil blend to a typical Western diet on lipids and inflammatory biomarkers	Non-smoking adults with LDL-C> 3·0 mmol/l BMI between 22 and 36 kg/m^2 LDL-C: 3.70 BMI: 28.6 No sample size calculations given	All food provided by researchers; no other food or beverages permitted. Western diet with fats from butter/olive oil/lard/sunflower oil; or high oleic canola oil or blend of high oleic canola oil and flaxseed oil Test diet composition calculated using food tables	Total-C: Vitros-350 chemistry analyser LDL-C: Friedewald 2.blood samples averaged	Controlled by cross-over design Weight assessed daily to ensure weight stability Adverse/other effects: HDL, TG, glucose	Total-C, LDL-C Diet 1: 5.7, 3.5 Diet 2: 5.3, 3.1 Diet 3: 5.1, 3.1 P<0.001 by ANOVA No carry-over effects were detected in plasma fatty acid concentrations
Iggman 2011	To compare the effect of replacing dairy fat with rapeseed (canola) oil on blood lipids, glucose metabolism and coagulation factors	Healthy adults, no smoking restriction LDL-C: 4.76 BMI: 26.3 Sample size was calculated based on lipid-lowering effects (not further described)	All food provided by researchers. Diet based on habitual Swedish diet with fats from butter/cream/high fat cheese or canola margarine. Test diet composition based on chemical analysis of the food	Total-C: IL Test Cholesterol Triander's method 181618-80 LDL-C: combination of preparative ultracentrifugation and precipitation	Controlled by cross-over design Weight stability maintained Adverse/other effects: HDL, TG, lipoprotein; glucose, insulin sensitivity, clotting factors	Total-C, LDL-C Diet 1: 6.7, 4.9 Diet 2: 5.6, 4.0 P<0.0001 No carry-over effects were detected
Marin 2011	To compare the effect of diets with different amounts of SFA, MUFA or carbohydrate on insulin sensitivity and the G972R polymorphism	Normolipidaemic (total-C<5.2mmol/L) college students; no smoking restriction BMI: 21-24 Post-hoc power analysis for the genotype/diet interaction	All food provided by researchers. Diets used butter/ palm oil or olive oil or CHO diet replaced some olive oil with biscuit, bread, jam, palm oil Test diet composition based on duplicate portion chemical analysis	Total-C: reference given LDL-C: Friedewald	Controlled by cross-over design Average weight constant across tests	Total-C, LDL-C (for predominant genotype) Diet 1: 3.6, 2.1 Diet 2: 3.7, 2.1 P<0.001 by ANOVA, apparently including the non-randomised SFA run-in

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Roussell 2012	To compare including lean beef in cholesterol lowering diets with similar SFA, PUFA, dietary cholesterol but varying amounts of lean beef with that of a healthy American diet on lipid responses	Non-smokers with elevated LDL-C (2.84-4.55mmol/L) BMI: 27.5 Sample size based on 9% reduction in LDL-C from original DASH diet, assuming power 80%, alpha 0.05, 2 tailed tests, with 10% dropout, n=40	All food provided by researchers. A DASH diet or a modified DASH diet with more protein supplied by lean beef (28 g vs 113 g respectively. Lean beef prepared without charring Test diet composition calculated using food tables	Total-C: Alfa Wassermann kit LDL-C: Friedewald	Controlled by cross-over design Weight assessed daily to ensure weight stability Adverse/other effects: HDL, TG, apolipoproteins, CRP, glucose, insulin	Total-C, LDL-C Diet 1: 5.0, 3.2 Diet 2: 5.0, 3.2 P: NS
Zhao 2004	To compare the effect of ALA, LA and an average American diet on multiple outcomes	Non-smoking, overweight adults with moderate hypercholesterolemia BMI: 28.1 No sample size calculations given	6-day cycle menu; walnuts, walnut oil and flaxseed oil used to modify fatty acid content of diet Unclear whether test diet composition was calculated or based on chemical analysis of the food	Total-C: method referenced LDL-C: Friedewald 2 blood samples averaged	Controlled by cross-over design Adverse/other effects: HDL, TG, CRP, markers of endothelial activation	Total-C, LDL-C Diet 1: 5.6, 3.7 Diet 2: 5.0, 3.3 Diet 3: 5.0, 3.3 P<0.05 pairwise comparisons against Diet 1 No carry-over effects were detected
Sabate 2003	To compare the effect of adding two different quantities of almonds to a Step 1 diet on multiple serum lipid values	Weight: 71 kg Sample size based on previous finding with nuts, with 10% dropout, n=24 would have >90% power for a significant (but unspecified) difference	All food provided by researchers; Almonds provided 10% or 20% energy; proportional reduction to all foods of Step 1 diet; almonds given on their own or in food Test diet composition based on duplicate portion chemical analysis	Total-C: Bayer 550 Express Chemistry analyzer LDL-C: Bayer 550 Express Chemistry analyzer 2.blood samples averaged	Controlled by cross-over design Weight assessed 2/week; weight maintained across test diets Adverse/other effects: HDL, TG, lipoproteins, glucose	Total-C, LDL-C Diet 1: 5.4, 3.7 Diet 2: 5.4, 3.7 Diet 3: 5.2, 3.5 P for trend: <0.001

Reference (First author, year of publication)	Objectives stated by author[@]	Participants & sample size	Interventions[@,#]	Methods of outcome measurement	Confounders	Results: concentrations at end of test diet - total-C/LDL-C (mmol/L)*
Curb 2000	To compare the effect of a diet rich in macadamia nuts with 37 %En from fat; a "typical American" diet with 37 %En from fat; and a Step 1 diet on lipid concentrations	Adults 80-130% ideal body weight BMI: 22-24 (M-F) No sample size calculations given	All food provided by researchers except Saturday evening meal. Up to 5 alcoholic beverages/week (except before blood sampling), 5 non-energy beverages per day allowed. Whole foods used to achieve macronutrient profiles; macadamia nuts were finely ground. Test diet composition based on chemical analysis of the food	Total-C: (Hitachi 717 Autoanalyzer LDL-C: Friedewald 3 blood samples averaged Measurements and laboratory analyses were performed blind	Controlled by cross-over design Weight assessed 2/week	Total-C, LDL-C Diet 1: 5.2, 3.4 Diet 2: 5.0, 3.2 Diet 3: 5.0, 3.2 P<0.01 pairwise comparisons against Diet 1 No carry-over effects were detected
Lacroix 2012	To compare the effect of a high but achievable intake of TFA from ruminant sources to a low-TFA butter and TFAs on plasma lipid concentrations	Healthy adults with a broad range of plasma LDL-C concentrations; no smoking restriction LDL-C: 2.84 BMI: 23.6 Sample size of 55 completers calculated assuming baseline LDL-C=3.0 mmol/L, >5% difference in LDL-C achieved, pooled SD of the change in LDL-C=0.39 mmol/L, 2-tailed alpha=0.05, beta=0.2.	Cows given corn oil to alter composition butter derived from their milk. This butter or oils used to vary TFA content while limiting variation in SFA content of diet. Diets were identical except for the test or a low-ruminant-TFA butter (both diets <2% TFA) All food provided by researchers; unlimited additional water, caffeine-free diet beverages permitted; tea and coffee restricted to 500mL/day. Test butters were laboratory analysed, total diet composition calculated using food tables		Controlled by cross-over design Weight assessed 5/week; weight 0.3kg different between diets Adverse/other effects: HDL, TG, lipoproteins, blood pressure	Total-C, LDL-C Diet 1: 5.2, 3.1 Diet 2: 5.2, 3.1 P: NS

[@] the objectives of the study include the authors overall goals and sometimes refer to goals and test diets which were not included in the review; the Interventions column only describes those test diets which were included in the review. The following test diet arms were excluded from the review because they did not adhere to the inclusion criteria given in Supporting Document 1:

- Hunter (2000): stearic rich diet had excess enrichment of an individual SFA
- Judd (2002): TFA, TFA+STE diets exceeded the TFA criteria for inclusion; LMP diet had excess enrichment of an individual SFA
- Vega-Lopez (2006): this study described an extension trial of Lichtenstein (1999) in a subset of subjects; the results for the two soybean arms had already been reported in Lichtenstein (1999) so were excluded here to avoid double-counting of data
- Lichtenstein (1999): Soft margarine, stick margarine, shortening diets exceeded the TFA criterion for inclusion; butter diet exceeded the allowed difference in dietary cholesterol between test arms
- Lovejoy (2002): T diet exceeded the TFA criterion for inclusion
- Castro (2000): Step 1 diet was the run-in and not part of the randomisation scheme
- Kris-Etherton (1999) Average American diet exceeded the allowed difference in dietary cholesterol between test arms
- Sanders (2003): Trans diet exceeded the TFA criterion for inclusion
- Kratz (2002): omega-3 PUFA diet - diets testing increase of long chain omega-3 were excluded
- Lichtenstein (2006): Hydrog-SO diet exceeded the TFA criteria for inclusion
- Motard-Belanger (2008): High ruminant TFA and industrial TFA diets exceeded the TFA criterion for inclusion
- Rajaram (2009): Salmon diet because diets testing increase of long chain omega-3 were excluded
- Marin (2011): SFA diet was the run-in and not part of the randomisation scheme
- Roussell (2012): HAD diet exceeded the allowed difference in dietary cholesterol between test arms; BOLD+ diet did not hold protein intake constant compared to other test arms

[#] Step 1 and Step 2 diets were previously recommended by the American Heart Association, but have been superseded by other dietary recommendations (Goodman et al. 1988). They contained different amounts fat and dietary cholesterol: Step 1 diet: <30 %En from total fat, <10 %En from SFA, <300 mg dietary cholesterol; Step II diet : <25 %En from total fat, <7 %En from SFA, <200 mg dietary cholesterol.

* The fatty acid composition of Diet 1, Diet 2 etc. is shown in Annex 3 of Supporting Document 1; p-value applies to both the total-C and LDL-C results unless otherwise stated

Appendix 2: GRADE summary of findings table

Overall Question: *Does decreasing saturated fat intake affect blood total or LDL cholesterol?*

Source: Supporting Document 1 and Mensink (2016)

A: Isoenergetic replacement of saturated fat (SFA) by carbohydrate (i.e. decrease in total fat intake)

Quality assessment of body of evidence							Participants	Mean effect estimate mmol/L (95% CI) per 1% energy exchange	Quality (degree of certainty in relationship)
Number of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Considerations**			
Decreased intake of SFA and isoenergetic replacement by carbohydrate and total cholesterol concentration									
74	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	2172	-0.041 (-0.047 to -0.035)	⊕⊕⊕⊕ High
Decreased intake of SFA and isoenergetic replacement by carbohydrate and LDL cholesterol concentration									
69	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	2026	-0.033 (-0.039 to -0.02)	⊕⊕⊕⊕ High

[1]Most studies were cross-over studies, at least single blind and provided all or the majority of foods to subjects and the test oil was used as an ingredient in food preparation, outcomes measured using standard laboratory methods, often auto-analysers

[2] The body of evidence was not down-rated for inconsistency because residual analysis supports a linear association; there was little variation about the regression line, studies have been conducted over more the 40 years in a range of countries and the additional studies included in the update did not change previous conclusions

[3]The body of evidence was not down-rated for indirectness because the outcomes were measured directly or, in the case of LDL-cholesterol, were calculated using the Friedewald equation which is the standard method; subjects with both normal cholesterol concentrations and elevated concentrations were studied.

[4]The body of evidence was not down-rated for imprecision because of the large number of studies and subjects, and the confidence intervals are narrow,

B: Isoenergetic replacement of saturated fat (SFA) by unsaturated fats (i.e. no change in total fat intake)

Quality assessment of body of evidence							Participants	Mean effect estimate mmol/L (95% CI) per 1% energy exchange	Quality (degree of certainty in relationship)
Number of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Considerations**			
Decreased intake of SFA and isoenergetic replacement by PUFA with 18 carbon atoms and total cholesterol concentration									
74	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	2172	-0.064 (-0.070 to -0.058)	⊕⊕⊕⊕ High
Decreased intake of SFA and isoenergetic replacement by PUFA with 18 carbon atoms and LDL cholesterol concentration									
69	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	2026	-0.055 (-0.061 to -0.050)	⊕⊕⊕⊕ High
Decreased intake of SFA and isoenergetic replacement by MUFA and total cholesterol concentration									
74	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	2172	-0.046 (-0.051 to -0.040)	⊕⊕⊕⊕ High
Decreased intake of SFA and isoenergetic replacement by MUFA and LDL cholesterol concentration									
69	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	2026	-0.042 (-0.047 to -0.037)	⊕⊕⊕⊕ High

[1]Most studies were cross-over studies, at least single blind and provided all or the majority of foods to subjects and the test oil was used as an ingredient in food preparation, outcomes were measured using standard laboratory methods, often auto-analysers

[2] The body of evidence was not down-rated for inconsistency because residual analysis supports a linear association; there was little variation about the regression line, studies have been conducted over more the 40 years in a range of countries and the additional studies included in the update did not change previous conclusions

[3]The body of evidence was not down-rated for indirectness because the outcomes were measured directly or, in the case of LDL-cholesterol, were calculated using the Friedewald equation which is the standard method; subjects with both normal cholesterol concentrations and elevated concentrations were studied.

[4]The body of evidence was not down-rated for imprecision because of the large number of studies and subjects, and the confidence intervals are narrow and exclude effects close to the null.

Overall Question: *Does increasing intake of LA or ALA affect serum total or LDL cholesterol?*

Quality assessment of body of evidence							Participants	Mean effect estimate mmol/L (95% CI) per 1% energy exchange	Quality (degree of certainty in relationship)
Number of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Considerations**			
Increased intake of LA as isoenergetic replacement of carbohydrate and total cholesterol concentration									
37	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	1125	-0.028 (-0.038 to -0.017)	⊕⊕⊕⊕ High
Increased intake of LA as isoenergetic replacement of carbohydrate and LDL-cholesterol concentration									
35	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	1041	-0.023 (-0.033 to -0.014)	⊕⊕⊕⊕ High
Increased intake of ALA as isoenergetic replacement of carbohydrate and total cholesterol concentration									
37	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	1125	-0.049 (-0.077 to -0.022)	⊕⊕⊕⊕ High
Increased intake of ALA as isoenergetic replacement of carbohydrate and LDL-cholesterol concentration									
35	RCTs	low[1]	none[2]	none[3]	none[4]	none to show	1041	-0.039 (-0.063 to -0.014)	⊕⊕⊕⊕ High

[1]Most studies were cross-over studies, at least single blind and provided all or the majority of foods to subjects and the test oil was used as an ingredient in food preparation, outcomes measured using standard laboratory methods, often auto-analysers
[2]The body of evidence was not down-rated for indirectness because the outcomes were measured directly or, in the case of LDL-cholesterol, were calculated using the Friedewald equation which is the standard method; subjects with both normal cholesterol concentrations and elevated concentrations were studied.
[3]The body of evidence was not down-rated for imprecision because of the large number of studies and subjects, and the confidence intervals are narrow and exclude effects close to the null.
[4]The body of evidence was not down-rated for inconsistency because the results for the individual fatty acids reflect the results of the class to which they belong