Cochrane Library

Cochrane Database of Systematic Reviews

Polyunsaturated fat intake for prevention of cardiovascular disease (Protocol)

Abdelhamid A, Martin N, Bridges C, Song F, Deane KHO, Hooper L

Abdelhamid A, Martin N, Bridges C, Song F, Deane KHO, Hooper L.
Polyunsaturated fat intake for prevention of cardiovascular disease.
Cochrane Database of Systematic Reviews 2016, Issue 9. Art. No.: CD012345.
DOI: 10.1002/14651858.CD012345.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Protocol]

Polyunsaturated fat intake for prevention of cardiovascular disease

Asmaa Abdelhamid[1], Nicole Martin[2], Charlene Bridges[2], Fujian Song[3], Katherine HO Deane[4], Lee Hooper[1]

[1]Norwich Medical School, University of East Anglia, Norwich, UK. [2]Farr Institute of Health Informatics Research, University College London, London, UK. [3]Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, UK. [4]Edith Cavell Building, University of East Anglia, Norwich, UK

Contact address: Lee Hooper, Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ, UK. l.hooper@uea.ac.uk.

Editorial group: Cochrane Heart Group.
Publication status and date: New, published in Issue 9, 2016.

Citation: Abdelhamid A, Martin N, Bridges C, Song F, Deane KHO, Hooper L. Polyunsaturated fat intake for prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews* 2016, Issue 9. Art. No.: CD012345. DOI: 10.1002/14651858.CD012345.

ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To assess the effects of altering total polyunsaturated fat intake on cardiovascular disease, and other health outcomes, in adults.

BACKGROUND

Description of the condition

The World Health Organization (WHO) reports cardiovascular diseases (CVDs) as the primary cause of death in the world (WHO 2016). In 2012 they estimated that 17.5 million people died from cardiovascular diseases, three-quarters of whom were in low- to middle-income countries. Cardiovascular diseases are disorders of the heart and blood vessels and include a range of conditions. Some are diseases of blood vessels supplying the heart (coronary heart disease), brain (cerebrovascular disease), or arms or legs (peripheral arterial disease). Others are due to infection (rheumatic heart disease, where damage to the heart muscle and valves is due to rheumatic fever), or are present at birth (congenital heart disease), or are due to blood clots (deep vein thrombosis and pulmonary embolism) (WHO 2016). This review is concerned with the forms of cardiovascular disease that are potentially modifiable by dietary means, particularly coronary heart disease and cerebrovascular disease.

Description of the intervention

Polyunsaturated fatty acids (PUFAs) are fats that include at least two double carbon-to-carbon bonds (unsaturated carbon bonds) in their long hydrocarbon chain. This makes the fats pack less well, so they tend to be liquid at room temperature, rather than solid like many saturated fats. PUFAs can be omega-3 (where the first double bond is three carbons away from the methyl-carbon end of the molecule), omega-6 or omega-9. Plant and fish oils are often rich in PUFAs, with fish being rich in omega-3 and plant oils rich in omega-6. Two PUFAs, alpha-linolenic acid (omega-3) and linoleic acid (omega-6), are essential nutrients in humans.
Dietary fats have been implicated in cardiovascular health since Keys published his groundbreaking study linking plasma cholesterol and dietary saturated fat (Keys 1950), and Oliver reported

higher levels of lower density lipid (LDL) cholesterol in those surviving myocardial infarction compared to controls without myocardial infarction (Oliver 1953). In 1965 Hegsted published an equation that quantified the relationship between dietary fat and serum total cholesterol, suggesting that increasing saturated fats increased serum cholesterol, while increasing PUFAs reduced serum cholesterol (Hegsted 1965). More recently there was discussion about what type of PUFA may be protective, with interest in omega-3 PUFAs following ground-breaking randomized controlled trials (RCTs) with dietary fish and fish oil supplementation interventions in the 1980s and 1990s(Burr 1989; GISSI-P 1999), although subsequent trials have been equivocal (Hooper 2004). Similarly, while there are good theoretical grounds for suggesting that omega-6 fats may be protective against cardiovascular diseases, the RCT evidence is limited (Al-Khudairy 2015). However, there is evidence that replacing saturated fats with PUFAs does protect against cardiovascular disease, and that PUFAs appear to be more protective than reducing saturated fats and replacing them with carbohydrates (Hooper 2015a). On the other hand, reducing dietary fat (including PUFAs) appears to result in lower weight in adults, suggesting that lower PUFA intake would tend to protect against cardiovascular disease (Hooper 2015b).

How the intervention might work

PUFAs are generally thought to work by producing a reduction in serum total and LDL cholesterol, which slows the progress of atherosclerosis (a complex syndrome in which plaque builds up inside the arteries over time, reducing blood flow and leading to an increased risk of blood clots), and so delays or prevents the onset of cardiovascular and cerebrovascular disease. This theory is reinforced by evidence that replacing saturated fats with polyunsaturated fats is associated with greater reductions in cardiovascular events and with greater serum total cholesterol reduction (Hooper 2015a). Additional modes of action have been proposed for omega-3 PUFAs, these include: lowering of blood pressure; reducing thrombotic tendency; anti-inflammatory and antiarrhythmic effects; improving vascular endothelial function; increasing plaque stability (through increased plaque calcification); and improving insulin sensitivity (Calder 2012; Ohwada 2016). Omega-6 PUFAs may reflect the general lipid-lowering effects of PUFAs, but there has been concern that high levels of omega-6 intake can increase production of 2-series prostaglandins and 4-series leukotrienes compared with the 3-series prostaglandins and 5-series leukotrienes associated with omega-3 intake. As the 2-series prostaglandins and 4-series leukotrienes exert a more potent proinflammatory effect, omega-6 could increase the risk of cardiovascular disease via this mechanism (Russo 2009).

Why it is important to do this review

The evidence on the health effects of total PUFA intake, which is the combination of omega-3 and omega-6 fats, is equivocal. As cardiovascular diseases are such important determinants of health, that place a particular burden on the poorest people (WHO 2016), it is vital that we understand the role of PUFAs in them so that we can provide the best advice for individuals and populations about how to eat to reduce the risk of ill health. This assessment of the health effects of total PUFA intake is needed alongside updated assessment of the effects of omega-3 and omega-6 fats specifically (Al-Khudairy 2015; Hooper 2004). This review will assess the overall impact of increasing total PUFA intake and will be interpreted in the light of effects on health of increasing omega-3 fats (in the planned update of Hooper 2004) and of increasing omega-6 fats (in the planned update of Al-Khudairy 2015).

OBJECTIVES

To assess the effects of altering total polyunsaturated fat intake on cardiovascular disease, and other health outcomes, in adults.

METHODS

Criteria for considering studies for this review

Types of studies

We will include randomized controlled trials (RCTs) that compare higher with lower polyunsaturated fat intakes, randomise at least 100 participants, and assess effects over at least 12 months. We will include studies reported as full-text, those published as abstracts only, and unpublished data. We will not include cross-over studies (as this design is inappropriate for outcomes such as cardiovascular disease events or mortality), but will include cluster-randomised studies, as long as there are at least six clusters.

Small trials that randomise fewer than 100 participants will be excluded due to concerns over small study bias and the consequent potential for random error to result in false positive conclusions (Roberts 2015).

Types of participants

We will include studies of adults (18 years of age and above), except those who are pregnant or acutely ill. Specifically, we will include studies of adults (18 years or older, men or women or both) at any risk of cardiovascular disease (with or without existing cardiovascular disease). Included participants can be adults with increased risk of cancer, those undergoing - or who have undergone - coronary artery bypass grafting or angioplasty, and those with

current or previous cardiovascular disease, nephritis in systemic lupus erythematosus, diabetes mellitus, rheumatoid arthritis, depression, cognitive impairment, or multiple sclerosis.

We will exclude participants who are pregnant or acutely ill (those with diagnosed current cancer, undergoing heart or renal transplantation, with HIV or AIDS, on haemodialysis, with immunoglobulin A (IgA) glomerulonephritis, or any other renal problem except in diabetes).

Where trials include some adults and some people under 18 years of age, then the study will be included if at least 90% of participants were aged 18 years or over at baseline, or where outcomes for adults can be separated from those for younger people.

Types of interventions

Eligible trials will compare higher with lower total polyunsaturated fat (PUFA) intakes. The intervention must be either dietary supplementation, or a provided diet, or advice on diet. The advice, foodstuffs or supplements must aim to increase or decrease total PUFA intake, or, if no clear aim is stated (but implied, such as aiming to provide a 'heart health' or 'Mediterranean' diet) then the intervention must achieve an increase or decrease of at least 10% of the baseline total PUFA level.

Supplementation may be in oil or capsule form, or as food-stuffs provided, to be consumed by mouth (we will exclude enteral and parenteral feeds, and enemas). Studies will not be included if they include a multiple risk factor intervention on lifestyle factors other than diet and supplementation (unless the effect of diet or supplementation could be separated out from the other interventions). Where the alteration of PUFA intake is only part of a dietary intervention (such as a combined intervention to increase PUFA and fruit and vegetable intake) the study will be included.

Studies will be included if they compared the effect of this intervention with usual diet, no advice, no supplementation or placebo (as appropriate) or with the opposite intervention (raised versus lowered PUFA intake).

Types of outcome measures

Primary outcomes

These outcomes have been requested by the WHO Nutrition Guidance Expert Advisory Group (NUGAG) to inform their planned dietary guidance. Dichotomous outcomes will be assessed at the latest point of available follow-up within the RCT while continuous outcomes will be assessed at one year and at the latest point in the trial.

Primary outcomes will be:

1. all-cause mortality;
2. major adverse cardiac and cerebrovascular events (MACCEs) or cardiovascular events: death, myocardial infarction, unstable angina or stroke;
3. coronary heart disease events: myocardial infarction (fatal or non-fatal) or angina;
4. stroke (total, ischaemic and haemorrhagic).

Secondary outcomes

Secondary outcomes will include:

1. cardiovascular mortality;
2. myocardial infarction;
3. sudden death;
4. angina;
5. heart failure;
6. peripheral vascular events;
7. atrial fibrillation;
8. revascularisation, angioplasty or coronary artery bypass grafting;
9. body weight and measures of adiposity;
10. type 2 diabetes;
11. serum lipids (including total cholesterol, fasting triglycerides, high-density lipid (HDL) cholesterol and LDL cholesterol);
12. blood pressure (systolic and diastolic);
13. quality of life measures (such as feelings of health and time off work);
14. economic costs;
15. serious adverse events (including breast cancer, all cancers, inflammatory bowel disease, neurocognitive outcomes such as dementia, and depression).

Search methods for identification of studies

Electronic searches

We will identify trials through systematic searches of the following bibliographic databases:

1. Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library;
2. MEDLINE (Ovid);
3. Embase (Ovid).

The preliminary search strategy for MEDLINE (Ovid) (Appendix 1) was adapted from the search strategy in Al-Khudairy 2015 and is also being used to update Al-Khudairy 2015. This complex strategy will be adapted for use in the other databases. The Cochrane sensitivity and precision-maximising RCT filter will be applied to MEDLINE (Ovid) and for Embase, and terms recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* will be applied (Lefebvre 2011).

We will also conduct a search of ClinicalTrials.gov (www.ClinicalTrials.gov) and the WHO International Clinical Trials Registry Platform (ICTRP) Search Portal (apps.who.int/trialsearch/).

We will search all databases from their inception to the present, and we will impose no restriction on language of publication.

Searching other resources

We will check reference lists of all included primary studies and relevant systematic reviews for additional references.

We will contact the authors of all RCTs that compare our intervention and control, randomise at least 100 participants, and follow them up for at least 12 months, to request available data on all of the review outcomes. Where data on at least one review outcome are available, the RCT will be included, and the authors asked to provide any additional data about study methodology or risk of bias.

Data collection and analysis

Selection of studies

Two review authors will independently screen titles and abstracts for inclusion of all the potential studies we identify as a result of the search and code them as 'retrieve' (eligible or potentially eligible/unclear) or 'do not retrieve'. If there are any disagreements, a third author will be asked to arbitrate (LH). We will retrieve the full-text study reports/publication and two review authors will independently screen the full-text, identify studies for inclusion, and identify and record reasons for exclusion of ineligible studies. We will resolve any disagreement through discussion or, if required, we will consult a third person (LH). We will identify and exclude duplicates and collate multiple reports of the same study so that each study - rather than each report - is the unit of interest in the review. We will record the selection process in sufficient detail to complete a PRISMA flow diagram and 'Characteristics of excluded studies' table.

Data extraction and management

We will use a data collection form for collating study characteristics and outcome data that has been piloted on at least one study in the review. Two review authors will extract the following study characteristics from included studies:

1. bibliographic details;
2. trial registration number;
3. methods: study design, total duration of study, details of any 'run in' period, number of study centres and location, study setting, withdrawals, and date of study;
4. participants: number randomized in each arm, number analyzed in each arm, mean age, age range, gender, health status, baseline cardiovascular disease risk, inclusion criteria, and exclusion criteria. Baseline cardiovascular risk will be categorised as low (no specific cardiovascular disease risk factors in the inclusion criteria), moderate (people recruited on the basis of hyperlipidaemia, diabetes, metabolic syndrome, familial risk, high blood pressure, obesity or a high cardiovascular disease risk score), or high (those with existing cardiovascular disease such as angina or a previous stroke or myocardial infarction);
5. interventions: intervention (including composition and dose of PUFA intake advised or supplement used), comparison, concomitant medications, and excluded medications;
6. outcomes: primary and secondary outcomes specified in trial registry, data on outcomes reported in publications and by contact with authors, time points reported;
7. process data: mean and standard deviation (SD) of total PUFA, omega-3, omega-6, total fat, saturated fat (SFA), monounsaturated fat (MUFA) and trans fat intake plus erythrocyte, serum or adipose tissue fatty acid status data in intervention and control groups at latest point available during RCT;
8. trial funding and notable conflicts of interest of trial authors.

Two review authors will independently extract outcome data from included studies. We will resolve disagreements by consensus or by involving a third person (LH). One review author will transfer data into the Review Manager 5 file (RevMan 2014). We will double-check that data are entered correctly by comparing the data presented in the systematic review with the study reports. A second review author will spot-check study characteristics for accuracy against the trial reports.

Assessment of risk of bias in included studies

Two review authors will independently assess risk of bias for each study, alongside data extraction, using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We will resolve any disagreements by discussion or by involving another author. We will assess the risk of bias according to the following domains:

1. random sequence generation;
2. allocation concealment;
3. blinding of participants and personnel;
4. blinding of outcome assessment;
5. incomplete outcome data;
6. selective outcome reporting;
7. industry funding;
8. preregistered on a trials register (registration date is before outcome data collection begins; Roberts 2015);
9. attention bias (another aspect of performance bias, where the intervention or control groups receive more time and attention from study or health personnel, or both, during the trial);
10. clear PUFA causality (another aspect of performance bias, where a study intervention included changes other than the change in PUFA intake, when there would be high risk of bias).

We will grade each potential source of bias as high, low or unclear and provide a quote from the study report together with a justification for our judgment in the 'Risk of bias' tables. We will summarize the risk of bias judgements across different studies for each of the domains listed. Where information on risk of bias relates to unpublished data or correspondence with a trialist, we will note this in the 'Risk of bias' tables.
When considering treatment effects, we will take into account the risk of bias for the studies that contribute to that outcome.

Assessment of bias in conducting the systematic review

We will conduct the review according to this published protocol and report any deviations from it in the 'Differences between protocol and review' section of the systematic review.

Measures of treatment effect

We will analyse dichotomous data as risk ratios with 95% confidence intervals and continuous data as mean differences (or standardised mean differences when different scales that measure the same effects are combined) with 95% confidence intervals. We will enter data presented as a scale with a consistent direction of effect. We will describe skewed data reported as medians and interquartile ranges narratively.

Unit of analysis issues

If we include cluster randomized trials we will account for unit of analysis issues by data extracting a direct estimate of the required effect measure (for example, a risk ratio with its confidence interval) from an analysis that accounts for the cluster design properly (for example, an analysis based on a 'multilevel model', a 'variance components analysis' or that uses 'generalised estimating equations (GEEs)'). If these data are available then we will use them in meta-analysis using the generic inverse-variance method (Higgins 2011). Where no such correct analysis of the cluster-randomised data are available, we will use approximate analyses using intracluster correlation co-efficient (ICC) analysis as outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011, section 16.3.4)

Dealing with missing data

We will contact investigators or study sponsors in order to verify key study characteristics, including those related to risk of bias. We will try to contact the authors of all included trials to request data on all relevant outcomes reported in their trial registry entry, or, where no trial registry entry can be found, we will ask for data on all of our review outcomes (primary and secondary). We will use this to obtain any missing numerical outcome data where possible.

Assessment of heterogeneity

We will use the I^2 statistic to measure heterogeneity among the trials in each analysis. If we identify substantial heterogeneity (assumed when I^2 is greater than 50%) we will report it and explore possible causes by prespecified subgroup analysis.

Assessment of reporting biases

If we are able to pool more than 10 trials, we will create and examine a funnel plot to explore possible reporting biases for the primary outcomes.

Data synthesis

We will undertake meta-analyses only where this is meaningful, i.e. if the treatments, participants and the underlying clinical question are similar enough for pooling to make sense. Our primary analyses will assess effects of dietary (dietary advice or supplemental foods such as fortified margarine provided by the study) or supplemental sources (capsules or oils) of total PUFA separately.
We will use a random-effects model, as dietary interventions are complex and somewhat heterogeneous by their nature (more so than most medical treatments), but we will compare the results of random-effects and fixed-effect meta-analysis in sensitivity analyses. As the random-effects model assigns more weight to smaller studies, it is more conservative and may lead to imprecise estimates of effect. However, as very small trials (randomising fewer than 100 participants) will not be included in this review, the risk of obscuring an important answer is minimised. We will also carry out sensitivity analyses to assess the effects of methodological rigour, as well as study size.

'Summary of findings' table

We will create a 'Summary of findings' table using the following outcomes:

1. all-cause mortality;
2. major adverse cardiac and cerebrovascular events;
3. coronary heart disease events;
4. stroke, total;
5. cardiovascular mortality;
6. type 2 diabetes;
7. serious adverse events (including breast cancer, all cancers, inflammatory bowel disease, neurocognitive outcomes such as dementia, and depression).

We will use the five GRADE considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the quality of a body of evidence as it relates to the studies that contribute data to the meta-analyses for the prespecified outcomes. We will use methods and recommendations described in Section 8.5 and Chapter 12 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011), and use

GRADEpro GDT software (GRADEpro GDT 2014). We will justify all decisions to downgrade the quality of studies using footnotes and we will make comments to aid reader's understanding of the review where necessary.

Subgroup analysis and investigation of heterogeneity

We will explore the effects of PUFA intake on primary outcomes by performing subgroup analyses on:

1. total PUFA dose (and dose response);
2. trial duration: studies with medium follow-up (12 to 23 months), medium to long follow-up (24 to 47 months), and long follow-up (48 months or more);
3. baseline total PUFA intake;
4. replacement of SFA with total PUFA;
5. replacement of MUFA with total PUFA;
6. for total PUFA: at least 6% of energy versus lower levels of intake, at least 11% of energy versus lower levels of intake;
7. PUFA dose response;
8. change in the omega-3:omega-6 ratio: does this intervention primarily increase omega-3 (putting up the ratio) or omega-6 (lowering the ratio)?
9. age;
10. sex;
11. baseline risk of cardiovascular disease.

We will use the formal test for subgroup interactions in Review Manager 5 (RevMan 2014).

For primary outcomes where sufficient trials (at least 10) are identified as suitable for meta-analysis, we will use meta-regression to explore the effects of PUFA dose, serum PUFA levels, baseline PUFA intake, omega-3:omega-6 ratio, supplemental or dietary source and trial duration.

Sensitivity analysis

We plan to carry out the following sensitivity analyses:

1. using fixed-effect meta-analysis;
2. only including studies with a low risk of bias for allocation concealment;
3. only including all studies up to 2010, plus studies post-2010 that are registered on a trials register (Roberts 2015);
4. only including studies with no industry funding;
5. only including studies with less than 10% difference in intake of trans fats between study arms during the intervention;
6. only including studies with a low risk of attention bias;
7. only including studies which randomized at least 250 participants.

Reaching conclusions

We will base our conclusions only on findings from the quantitative or narrative synthesis of included studies for this review. We will avoid making recommendations for practice and our implications for research will suggest priorities for future research and outline what the remaining uncertainties are in the area.

ACKNOWLEDGEMENTS

Thank you to Charlene Bridges for designing and carrying out the searches, to Nicole Martin and Juan-Pablo Casas for discussions on the protocol, and to all Cochrane Heart Group staff and editors for fast and helpful comments and support. Thank you also to the World Health Organization for commissioning and funding the review.

REFERENCES

Additional references

Al-Khudairy 2015
Al-Khudairy L, Hartley L, Clar C, Flowers N, Hooper L, Rees K. Omega 6 fatty acids for the primary prevention of cardiovascular disease. *Cochrane Database of Systematic Reviews* 2015, Issue 11. [DOI: 10.1002/14651858.CD011094.pub2]

Burr 1989
Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989;**2**(8666):757–61.

Calder 2012
Calder PC. Mechanisms of action of (n-3) fatty acids. *Journal of Nutrition* 2012;**142**(3):592S–9S. [DOI: 10.3945/%E2%80%8Bjn.111.155259]

GISSI-P 1999
GISSI-P: Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;**354**(9177):447–55.

GRADEpro GDT 2014 [Computer program]
GRADE Working Group, McMaster University. GRADEpro GDT. Version (accessed 26 August 2016). Hamilton (ON): GRADE Working Group, McMaster University, 2014.

Hegsted 1965
Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. *American Journal of Clinical Nutrition* 1965;**17**(5):281–95.

Higgins 2011
Higgins JPT, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Hooper 2004
Hooper L, Thompson RL, Harrison RA, Summerbell CD, Moore H, Worthington HV, et al. Omega 3 fatty acids for prevention and treatment of cardiovascular disease. *Cochrane Database of Systematic Reviews* 2004, Issue 4. [DOI: 10.1002/14651858.CD003177.pub2]

Hooper 2015a
Hooper L, Martin N, Abdelhamid A, Davey Smith G. Reduction in saturated fat intake for cardiovascular disease. *Cochrane Database of Systematic Reviews* 2015, Issue 6. [DOI: 10.1002/14651858.CD011737]

Hooper 2015b
Hooper L, Abdelhamid A, Bunn D, Brown T, Summerbell CD, Skeaff DM. Effects of total fat intake on body weight. *Cochrane Database of Systematic Reviews* 2015, Issue 8. [DOI: 10.1002/14651858.CD011834]

Keys 1950
Keys A, Mickelsen O, Miller EVO, Carleton B. The relation in man between cholesterol levels in the diet and in the blood. *Science* 1950;**112**:79–81.

Lefebvre 2011
Lefebvre C, Manheimer E, Glanville J. Chapter 6: Searching for studies. In: Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Ohwada 2016
Ohwada T, Yokokawa T, Kanno Y, Hotsuki Y, Sakamoto T, Watanabe K, et al. Vascular composition data supporting the role of N-3 polyunsaturated fatty acids in the prevention of cardiovascular disease events. *Data Brief* 2016;**7**:1237–47. [DOI: http://dx.doi.org/10.1016/j.dib.2016.03.101]

Oliver 1953
Oliver MF, Boyd GS. The plasma lipids in coronary artery disease. *British Heart Journal* 1953;**15**:387–90.

RevMan 2014 [Computer program]
The Cochrane Collaboration. Review Manager 5 (RevMan 5). Version 5.3. Copenhagen: The Nordic Cochrane Centre: The Cochrane Collaboration, 2014.

Roberts 2015
Roberts I, Ker K, Edwards P, Beecher D, Manno D, Sydenham E. The knowledge system underpinning healthcare is not fit for purpose and must change. *BMJ* 2015;**350**:h2463. [DOI: http://dx.doi.org/10.1136/bmj.h2463]

Russo 2009
Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochemical Pharmacology* 2009;**77**(6):937–46.

WHO 2016
World Health Organization. Cardiovascular diseases (CVDs). Fact sheet number 317. www.who.int/mediacentre/factsheets/fs317/en/ reviewed June 2016: (accessed 26 July 2016).

* *Indicates the major publication for the study*

APPENDICES

Appendix 1. Preliminary MEDLINE (Ovid) search strategy

1. exp fatty acids, essential/
2. fatty acids, unsaturated/
3. ((polyunsaturat* or poly-unsaturat*) adj3 fat*).ti,ab.
4. (poly* adj4 unsat* adj4 fatty acid*).ti,ab.
5. PUFA.ti,ab.
6. exp fatty acids, omega-6/
7. omega-6.ti,ab.
8. (n-6 adj4 acid*).ti,ab.
9. linoleic acid*.ti,ab.
10. corn oil/ or cottonseed oil/ or olive oil/ or safflower oil/ or sesame oil/ or soybean oil/
11. ((corn or maize or mazola) adj4 oil*).ti,ab.
12. (cottonseed* or (cotton adj seed*)).ti,ab.
13. (olive adj4 oil*).ti,ab.
14. (safflower adj4 oil*).ti,ab.

15. (sesame adj4 oil*).ti,ab.
16. ((soy bean or soybean) adj4 (oil* or fat*)).ti,ab.
17. (so?a adj4 oil*).ti,ab.
18. so?aoil*.ti,ab.
19. (soy adj4 oil*).ti,ab.
20. (sunflower adj4 oil*).ti,ab.
21. helianth*.ti,ab.
22. (grapeseed adj4 oil*).ti,ab.
23. (canola adj4 oil*).ti,ab.
24. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23
25. randomized controlled trial.pt.
26. controlled clinical trial.pt.
27. randomized.ab.
28. placebo.ab.
29. clinical trials as topic.sh.
30. randomly.ab.
31. trial.ti.
32. 25 or 26 or 27 or 28 or 29 or 30 or 31
33. exp animals/ not humans.sh.
34. 32 not 33
35. 24 and 34

CONTRIBUTIONS OF AUTHORS

Conceiving and designing the review: LH

Discussion and drafting of the protocol: Lee Hooper (LH), Asmaa Abdelhamid (AA), Fujian Song (FS), Katherine Deane (KD), Nicole Martin (NM), Charlene Bridges (CB)

Co-ordinating the protocol and review: LH

DECLARATIONS OF INTEREST

AA: This review was funded by a grant from the World Health Organization.

NM: None known.

CB: None known.

FS: This review was funded by a grant from the World Health Organization.

KD: This review was funded by a grant from the World Health Organization.

LH: This review was funded by a grant from the World Health Organization.

SOURCES OF SUPPORT

Internal sources

- University of East Anglia, UK.

External sources

- The Cochrane Heart Group US Satellite is supported by intramural support from the Northwestern University Feinberg School of Medicine and the Northwestern University Clinical and Translational Science (NUCATS) Institute (UL1TR000150)., USA.
- This project was supported by the National Institute for Health Research, via Cochrane Infrastructure to the Heart Group. The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Systematic Reviews Programme, NIHR, NHS or the Department of Health, UK.
- World Health Organization, Switzerland.

Funding to carry out this systematic review came from the World Health Organization