Omega 6 fatty acids for the primary prevention of cardiovascular disease (Protocol)

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TABLE OF CONTENTS

[Intervention Protocol]

Omega 6 fatty acids for the primary prevention of cardiovascular disease

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ABSTRACT

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

The two primary objectives of this review are to:

1. determine the effectiveness of increasing omega 6 (LA, GLA, DGLA, AA, or any combination) intake in place of saturated or monounsaturated fats or carbohydrates for the primary prevention of CVD;

2. determine the effectiveness of decreasing omega 6 (LA, GLA, DGLA, AA, or any combination) intake in place of carbohydrates or protein (or both) for the primary prevention of CVD.

BACKGROUND

Description of the condition

Cardiovascular diseases (CVD) are a group of conditions that affect the heart and blood vessels (WHO 2013), and include cerebrovascular disease, coronary heart disease (CHD), and peripheral arterial disease (PAD). One mechanism thought to cause CVD is atherosclerosis, which is where arteries become blocked by plaques or atheromas (NHS 2012). Atherosclerosis can cause CVD when the arteries are completely blocked by a blood clot or when a narrowed artery restricts blood flow limiting the amount of blood and oxygen reaching organs or tissue (BHF 2013). Even though arteries may narrow and become less elastic with age, the process may be accelerated by factors such as smoking, high cholesterol, hypertension, obesity, a sedentary lifestyle, and ethnicity (NHS 2012). Ruptures of unstable plaques may also cause CVD by activating an inflammatory response in the body. This inflammatory response causes the structure of the atherosclerotic plaque to weaken and rupture leading to the formation of blood clots (Spagnoli 2007). CVDs are the leading causes of death worldwide (WHO 2013), and in 2008 an estimated 30% of all global deaths were due to CVD (WHO 2013). The burden of CVD also varies substantially between regions (Müller-Nordhorn 2008), for example, death from ischaemic heart disease in France is one-quarter of that of the UK (Law 1999). Furthermore, low- and middle-income countries are disproportionally affected (WHO 2013): in 2001, three-quar-

ters of global deaths from CHD took place in low- and middle-income countries (Gaziano 2010). Gaziano et al. suggest that this rapid increase in CHD burden is attributable to an increase in life span, socioeconomic changes, and the acquisition of lifestyle-related risk factors (Gaziano 2010).

One key public health priority is targeting modifiable risk factors for CVD prevention (e.g. dietary factors). Such risk factors are important since their modification has the potential to lower CVD risk making them a main target for interventions aimed at CVD primary prevention. One major modifiable risk factor is diet and dietary factors, such as a low consumption of fruit and vegetables (Begg 2007), a high intake of saturated fat (Siri-Tarino 2010), and a high consumption of salt (He 2010), have been found to be associated with CVD risk.

Description of the intervention

Omega 6 (or n-6) polyunsaturated fatty acids (PUFA) are characterised by the presence of at least two carbon-carbon double bonds (Harris 2009), as are omega 3 fats. However, they differ from omega 3 in the position of this double bond from the methyl group end of the chain (Calder 2013; Hall 2009). For omega 3, the carbon-carbon double bond is third from the methyl terminus whereas for omega 6 the double bond pair is sixth from the methyl terminus (Calder 2013; Harris 2009). One of the omega 6 fatty acids, linoleic acid (LA) (18:2n-6), is not synthesized in the body and so has to be obtained via the diet (Groff 1995). It is an essential fatty acid (the other essential fatty acid is alpha-linolenic acid (ALA), an omega 3 fatty acid). Another omega 6 fatty acid, gamma-linolenic acid (GLA), is conditionally essential (essential in some conditions, but not usually). The remaining omega 6 fatty acids are not essential, as the body can make them from other fatty acids.

LA is an omega 6 short-chain PUFA, while long-chain omega 6 PUFA, that can be created from LA in the healthy adult human body, include GLA (18:3n-6), dihomo-gamma-linolenic acid (DGLA) (20:3n-6), and arachidonic acid (AA) (20:4n-6). LA is widely available in the diet from a variety of vegetable oils, nuts and nut oils, poultry, meat, egg, milk, margarines, and spreads (Russo 2009). In a Swedish population of 50-year old men, LA made up 54% as a proportion of serum cholesteryl esters, while GLA and DGLA were each less than 1% and AA was 5% (approximately 60% of cholesteryl esters were of the omega 6 family, compared with less than 3% omega 3 fatty acids, 14% saturated fats and 23% monounsaturated fats (Warensjo 2008). In the USA, the American Heart Association (AHA) in 2010 recommended an omega 3 intake of around 500 mg per day (from food or capsules) and about 15 mg per day of LA (Harris 2010).

As with omega 3, omega 6 fatty acids play a vital role in many physiological functions. They are particularly important for maintaining bone health, regulating metabolism, and in stimulating skin and hair growth. However, omega 6 fatty acids are controversial in their effect on cardiovascular risk. Some evidence suggests that a proportionally higher intake of omega 6 fatty acids along with a low intake of saturated fat is associated with significant reductions in CHD (Katan 2009). Indeed, evidence from cohort studies have shown omega 6 fatty acids to be inversely associated with cardiovascular death and to be inversely associated with CHD risk (Laaksonen 2005; Oh 2005). Furthermore, evidence has shown omega 6 fatty acids to reduce total and low-density lipoprotein (LDL) cholesterol (Hodson 2001; Jakobsen 2009), and also decrease blood pressure (Hall 2009). In contrast, there is concern that high levels of omega 6 fatty acids, compared with omega 3 fatty acids, in the diet increase the production of 2-series prostaglandins and 4-series leukotrienes. These exert a more potent proinflammatory effect than the 3-series prostaglandins and 5-series leukotrienes from omega 3. In doing this, they may potentially worsen cardiovascular risk (Russo 2009).

How the intervention might work

Atypical lipoprotein levels and hypertension are major risk factors for atherosclerosis, a major cause of CVD (Briel 2009). Dietary and lifestyle interventions to modify serum lipids and blood pressure can in turn modify cardiovascular risk. Omega 6 fatty acids are controversial in their effect on cardiovascular risk. One systematic review of metabolic replacements for saturated fat suggests that PUFAs (of which LA is a major component), reduce LDL cholesterol more than replacements by carbohydrate or monounsaturated fats (Mensink 2003). However, there is concern that high levels of omega 6 fatty acids, compared with omega 3 fatty acids, in the diet increase the production of 2-series prostaglandins and 4-series leukotrienes compared with 3-series prostaglandins and 5-series leukotrienes. As the 2-series prostaglandins and 4-series leukotrienes exert a more potent proinflammatory effect, omega 6 fatty acids may theoretically worsen cardiovascular risk (Russo 2009). This relationship is disputed, but has led to the concept that the ratio between omega 6 and omega 3 fatty acids may be crucial, rather than absolute intakes of either omega 6 or omega 3 fatty acids. In addition, there is concern that highly unsaturated fatty acids such as AA may increase the susceptibility of lipoproteins such as LDL and very-low-density lipoproteins (VLDL) to oxidation, making them more atherogenic (Russo 2009).

One cross-sectional Japanese study suggested that higher LA intake was associated with lower concentrations of atherogenic LDL cholesterol and of large VLDL particles, and with higher levels of large high-density lipoprotein (HDL) particles (Choo 2010), all of which associate higher LA intake with lower cardiovascular risk. One cross-sectional study of 4680 men and women aged 40 to 59 years from China, Japan, the US, and the UK found that higher LA intake was associated with significantly lower systolic and diastolic blood pressure (Miura 2008). One prospective cohort study in 1885 men aged 50 years with a 33.7-year follow-up found that higher LA as a proportion of serum cholesteryl esters was associ-

ated with a decrease in overall mortality and CVD risk (Warensjo 2008). Other studies have shown no association between omega 6 intake and CVD risk (O'Sullivan 2011; Rhee 2008).

One meta-analysis of observational studies suggested an inverse relationship between omega 6 intake and CVD risk (Harris 2007). The meta-analysis aimed to evaluate studies assessing the relationship between blood/tissue omega 6 PUFA content and CHD events, and was based on 25 case-control studies with 1998 cases and 6913 controls. Harris et al. found that LA content of blood and tissues was inversely associated with CHD risk; however, AA was not related to CHD risk. However, all of these observational findings should be treated with caution because of the potential for confounding effects by factors commonly associated with high omega 6 intake, such as healthier lifestyles, which might contribute to the observed inverse associations found.

One meta-analysis that searched for controlled intervention trials to 1999 included 1672 trials that found that omega 6 PUFAs had a beneficial effect on blood lipid levels (Mensink 2003). This was supported by one meta-analysis of eight randomised controlled trials (RCTs) (13,614 participants) investigating the effects of replacing saturated fatty acids with increased PUFA intake (including omega 6 and omega 3 fatty acids) on CHD end points. The meta-analysis found a reduction in CHD events when replacing SFA intake with PUFA (Mozaffarian 2010). One systematic review of RCTs that aimed to reduce or modify dietary fats found that modification of dietary fat (replacing saturated fats by monounsaturated fatty acids or PUFAs) reduced cardiovascular events (although there was no good evidence of reductions in total mortality or cardiovascular deaths), while replacing saturated fats with carbohydrate was not helpful (Hooper 2011).

Why it is important to do this review

There appears to be inconclusive evidence from observational studies and meta-analyses about the benefit of omega 6 intake on CVD outcomes. Therefore, an up-to-date systematic review is needed in order to clarify the association between CVD risk and omega 6 intake. This can then provide guidance for national and international agencies, practitioners, and members of the public.

This current review will also update and expand the most recent systematic reviews (Mensink 2003; Mozaffarian 2010). We will examine all RCTs stating an intention to increase or decrease omega 6 fats by following dietary advice, omega 6 supplementation, or a provided diet. We will examine the effects over long time periods (at least six months) as these are most relevant for public health interventions.

OBJECTIVES

The two primary objectives of this review are to:

1. determine the effectiveness of increasing omega 6 (LA, GLA, DGLA, AA, or any combination) intake in place of saturated or monounsaturated fats or carbohydrates for the primary prevention of CVD;

2. determine the effectiveness of decreasing omega 6 (LA, GLA, DGLA, AA, or any combination) intake in place of carbohydrates or protein (or both) for the primary prevention of CVD.

METHODS

Criteria for considering studies for this review

Types of studies

We will include RCTs. We will include studies reported as full-text, as abstract only, and unpublished data.

Types of participants

Healthy adults (aged 18 years old or over) from the worldwide general population and adults at moderate to high risk of CVD. As the review will focus on the primary prevention of CVD, we will exclude people who have experienced a myocardial infarction (MI), stroke, revascularisation procedure (coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA)), people with angina, and people with angiographically defined CHD. We will also exclude people with type 2 diabetes, as, while type 2 diabetes is a risk factor for CVD, interventions targeting this condition are covered by the Cochrane Metabolic and Endocrine Disorders Group.

Types of interventions

All RCTs of interventions stating an intention to increase or decrease omega 6 fatty acids. Interventions have to involve dietary advice, supplementation, or provide a diet where omega 6 fatty acids are either increased or decreased. Studies can include any type of omega 6 or combination of omega 6 fatty acids. We will consider trials involving an increase or decrease in omega 6 fatty acids with energy replacement by carbohydrates, omega 3, omega 9, saturated fats, protein, alcohol, or monounsaturated fats, and will include studies with additional dietary interventions. We will not include trials involving exercise.

We will also focus on follow-up periods of six months (24 weeks) or more. Follow-up is considered to be the time elapsed since the start of the intervention and, therefore, we will exclude any trials with an intervention period of less than six months. We will also consider trials where the comparison group is given no advice, no

supplementation, a placebo, a control diet, or continues with their usual diet.

Types of outcome measures

Primary outcomes

1. All-cause mortality.
2. Cardiovascular mortality.
3. Non-fatal end points such as MI, CABG, PTCA, angina, angiographically defined CHD, stroke, carotid endarterectomy, and PAD.

Secondary outcomes

1. Changes in blood pressure (systolic and diastolic blood pressure) and blood lipids (total cholesterol, HCL cholesterol, LDL cholesterol, triglycerides).
2. Occurrence of type 2 diabetes as a major CVD risk factor.
3. Adverse effects (as defined by the authors of the included trials).

Search methods for identification of studies

Electronic searches

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

- Cochrane Central Register of Controlled Trials (CENTRAL, *The Cochrane Library*);
- MEDLINE (Ovid);
- EMBASE (Ovid);
- CINAHL (EBSCO);
- ISI Web of Science;
- Database of Abstracts of Reviews of Effects (DARE), Health Technology Assessment Database, and Health Economics Evaluations Database on *The Cochrane Library*;
- AMED.

We will adapt the preliminary search strategy for MEDLINE (Ovid) for use in the other databases (Appendix 1). We will apply the Cochrane sensitivity-maximising RCT filter (Lefebvre 2011) to MEDLINE (Ovid) and adaptations of it to the other databases, except CENTRAL.
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 primary studies and review articles for additional references. We will search relevant manufacturers' websites for trial information.
We will also conduct a search of ClinicalTrials.gov (www.clinicaltrials.gov), metaRegister of controlled trials (mRCT) (www.controlled-trials.com/mrct), and the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) Search Portal (apps.who.int/trialsearch/).
We will perform citation searches on key articles and will use Google Scholar to search for further studies. We will contact experts in the field for unpublished and ongoing trials and study authors where necessary for any additional information.

Data collection and analysis

Selection of studies

Two authors (LH, NF) will independently screen titles and abstracts for inclusion of all the potential studies identified 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 arbitrate (KR). We will retrieve the full-text reports/publications and two authors (LH, NF) will independently screen the full-text and identify studies for inclusion, and identify and record reasons for exclusion of the ineligible studies. We will resolve any disagreement through discussion or, if required, we will consult a third author (KR). 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 details are available to complete a PRISMA flow diagram and 'Characteristics of excluded studies' table.

Data extraction and management

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

1. 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.
2. Participants: number, mean age, age range, gender, severity of condition, diagnostic criteria, baseline lung function, smoking history, inclusion criteria, and exclusion criteria.
3. Interventions: intervention, comparison, concomitant medications, and excluded medications.
4. For intervention and control (during intervention): the percentage of energy from omega 3, omega 6, omega 9, saturated

fats, monounsaturated fats, carbohydrates (refined and unrefined if possible), alcohol, protein, and omega 6/omega 3 ratio.

5. Outcomes: primary and secondary outcomes specified and collected, and time points reported.

6. Notes: funding for trial, and notable conflicts of interest of trial authors.

Two authors (LH, NF) will independently extract outcome data from included studies. We will resolve disagreements by consensus or by involving a third author (KR). One author (LH) will transfer data into Review Manager 5 (RevMan 2012). We will double-check that data are entered correctly by comparing the data presented in the systematic review with the data in the study reports. A second author (NF) will spot-check study characteristics for accuracy against the trial reports.

Assessment of risk of bias in included studies

Two authors (LH, NF) will independently assess risk of bias for each study 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 (KR). 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. Other bias. (e.g. industry funding).

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 judgement in the 'Risk of bias' table. We will summarise 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' table.

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 odds ratios or risk ratios with 95% confidence intervals and continuous data as mean difference or standardised mean difference with 95% confidence intervals. We will enter data presented as a scale with a consistent direction of effect.

We will narratively describe skewed data reported as medians and interquartile ranges.

Unit of analysis issues

Studies with multiple intervention groups

Data for the control group will be used for each intervention group comparison. We will reduce the weight assigned to the control group by dividing the number of participants in the control group by the number of intervention groups.

Cluster randomised trials

We will analyse cluster randomised trials using the unit of randomisation (cluster) as the number of observations. We will use individual level means and standard deviations adjusted for clustering together with the number of clusters in the denominator, where needed, in order to weight the trials appropriately.

Dealing with missing data

We will contact investigators or study sponsors in order to verify key study characteristics and obtain missing numerical outcome data where possible (e.g. when a study is identified as abstract only). Where this is not possible, and the missing data are thought to introduce serious bias, we will explore the impact of including such studies in the overall assessment of results using a sensitivity analysis.

Assessment of heterogeneity

We will use the I^2 statistic to measure heterogeneity among the trials in each analysis. If we identify substantial heterogeneity (heterogeneity of greater than 50%), we will report it and explore possible causes by pre-specified 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 small-study biases for the primary outcomes.

Data synthesis

We will conduct statistical analysis using Review Manager 5 (RevMan 2012). We will enter dichotomous data as events and the number of participants and continuous data as means and standard deviations. In the absence of substantial heterogeneity

(greater than 50%) and provided that there is a sufficient number of trials, we will combine the results using a fixed-effect model.

Subgroup analysis and investigation of heterogeneity

We plan to carry out the following subgroup analyses.

1. Omega 6 alone versus omega 6 plus other dietary components.
2. Baseline risk.
3. Increase or decrease in omega 6.
4. Omega 6/omega 3 ratio.
5. Age.
6. Sex.

We will also assess the treatment effects of omega 6 according to energy replacement (e.g. by what omega 6 is replacing or, for a decrease in omega 6, what is replacing omega 6). These will include:

1. carbohydrates;
2. saturated fats;
3. omega 3 fatty acids;
4. omega 9 fatty acids;
5. protein;
6. alcohol;
7. monounsaturated fats.

We will further explore the effects of the above different energy replacements by using meta-regression on our primary outcomes. We will use the formal test for subgroup interactions in Review Manager 5 (RevMan 2012).

Sensitivity analysis

We plan to carry out the following sensitivity analyses.

1. Only including studies with a low risk of bias (studies are assessed using The Cochrane Collaboration 'Risk of bias' tool).
2. Only including studies where the alteration in omega 6 (and its energy replacement) is the only dietary intervention.

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.

REFERENCES

Additional references

Begg 2007
Begg S, Vos T, Barker B, Stevenson C, Stanley L, Lopez A. The burden of disease and injury in Australia 2003. www.aihw.gov.au/publication-detail/?id=6442467990 (accessed 24 April 2014).

BHF 2013
British Heart Foundation. Cardiovascular disease. www.bhf.org.uk/heart-health/conditions/cardiovascular-disease.aspx (accessed 24 April 2014).

Briel 2009
Briel M, Ferreira-Gonzalez I, You JJ, Karanicolas PJ, Akl EA, Wu P, et al.Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: systematic review and meta-regression analysis. *BMJ* 2009;**16**(338):1–8.

Calder 2013
Calder P. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?. *British Journal of Clinical Pharmacology* 2013;**75**(3):645–62.

Choo 2010
Choo J, Ueshima H, Curb JD, Shin C, Evans RW, El-Saed A, et al.Serum n-6 fatty acids and lipoprotein subclasses in middle-aged men: the population-based cross-sectional ERA-JUMP study. *American Journal of Clinical Nutrition* 2010;**91**(5):1195–203.

Gaziano 2010
Gaziano TA, Bitton A, Anand S, Abrahams-Gessel S, Murphy A. Growing epidemic of coronary heart disease in low- and middle-income countries. *Current Problems in Cardiology* 2010;**35**(2):72–115.

Groff 1995
Groff JL, Gropper SS, Hunt SM. *Advanced Nutrition and Human Metabolism*. New York: West Publishing Company, 1995.

Hall 2009
Hall WL. Dietary saturated and unsaturated fats as determinants of blood pressure and vascular function. *Nutrition Research Reviews* 2009;**22**:18–38.

Harris 2007
Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* 2007;**193**(1):1–10.

Harris 2009
Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, et al.Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation* 2009;**119**(6):902–7.

Harris 2010
Harris W. Omega-6 and omega-3 fatty acids: partners in prevention. *Current Opinion in Clinical Nutrition and*

Metabolic Care 2010;**13**(2):125–9.

He 2010
He FJ, MacGregor GA. Reducing population salt intake worldwide: from evidence to implementation. *Progress in Cardiovascular Diseases* 2010;**52**(5):363–82.

Higgins 2011
Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Hodson 2001
Hodson L, Skeaff CM, Chisholm WA. The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. *European Journal of Clinical Nutrition* 2001;**55**: 908–15.

Hooper 2011
Hooper L, Summerbell CD, Thompson R, Sills D, Roberts FG, Moore H, et al.Reduced or modified dietary fat for preventing cardiovascular disease. *Cochrane Database of Systematic Reviews* 2011, Issue 7. [DOI: 10.1002/14651858.CD002137.pub2]

Jakobsen 2009
Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, et al.Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *American Journal of Clinical Nutrition* 2009;**89**: 1425–32.

Katan 2009
Katan MB. Omega-6 polyunsaturated fatty acids and coronary heart disease. *American Journal of Clinical Nutrition* 2009;**89**(5):1283–4.

Laaksonen 2005
Laaksonen DE, Nyyssönen K, Niskanen L, Rissanen TH, Salonen JT. Prediction of cardiovascular mortality in middle-aged men by dietary and serum linoleic and polyunsaturated fatty acids. *Archives of Internal Medicine* 2005;**165**:193–99.

Law 1999
Law M, Wald N. Why heart disease mortality is low in France: the time lag explanation. *BMJ* 1999;**318**:1471–80.

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

Mensink 2003
Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American Journal of Clinical Nutrition* 2003;**77**(5):1146–55.

Miura 2008
Miura K, Stamler J, Nakagawa H, Elliott P, Ueshima H, Chan Q, et al.Relationship of dietary linoleic acid to blood pressure. The International Study of Macro-Micronutrients and Blood Pressure Study. *Hypertension* 2008;**52**(2): 408–14.

Mozaffarian 2010
Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: a systematic review and meta-analysis of randomized controlled trials. *PLoS Medicine* 2010;**7**(3): e1000252.

Müller-Nordhorn 2008
Müller-Nordhorn J, Binting S, Roll S, Willich S. An update on regional variation in cardiovascular mortality within Europe. *European Heart Journal* 2008;**29**:1316–26.

NHS 2012
National Health Service. Atherosclerosis, 2012. www.nhs.uk/conditions/Atherosclerosis/Pages/Introduction.aspx#commentCountLink (accessed 24 April 2014).

O'Sullivan 2011
O'Sullivan TA, Bremner AP, Beilin LJ, Ambrosini GL, Mori TA, Huang RC, et al.Polyunsaturated fatty acid intake and blood pressure in adolescents. *Journal of Human Hypertension* 2011;**26**(3):178–87.

Oh 2005
Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC. Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. *American Journal of Epidemiology* 2005;**161**:672–79.

RevMan 2012
The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). 5.2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012.

Rhee 2008
Rhee Y, Paik MJ, Kim KR, Ko YG, Kang ES, Cha BS, et al.Plasma free fatty acid level patterns according to cardiovascular risk status in postmenopausal women. *Clinica Chimica Acta* 2008;**392**(1-2):11–6.

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.

Siri-Tarino 2010
Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *American Journal of Clinical Nutrition* 2010;**91**:535–46.

Spagnoli 2007
Spagnoli LG, Bonanno E, Sangiorgi G, Mauriello A. Role of inflammation in atherosclerosis. *Journal of Nuclear Medicine* 2007;**48**(11):1800–15.

Warensjo 2008

Warensjo E, Sundstrom J, Vessby B, Cederholm T, Riserus U. Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: a population based prospective study. *American Journal of Clinical Nutrition* 2008;**88**(1):203–9.

WHO 2013

World Health Organization. Cardiovascular diseases (CVDs). Fact sheet number 317. www.who.int/mediacentre/factsheets/fs317/en/ (accessed 24 April 2014).

* *Indicates the major publication for the study*

APPENDICES

Appendix 1. Preliminary search strategy - MEDLINE (Ovid)

1. Fatty Acids, Omega-6/
2. omega 6.tw.
3. (n-6 adj4 acid*).tw.
4. (n 6 adj4 acid*).tw.
5. omega-6.tw.
6. linoleic acid*.tw.
7. (poly* adj4 unsat* adj4 fatty acid*).tw.
8. PUFA.tw.
9. Dietary Fats, Unsaturated/
10. corn oil/
11. ((corn or maize or mazola) adj4 oil*).tw.
12. maydol.tw.
13. lipomul*.tw.
14. cottonseed oil/
15. cottonseed*.tw.
16. cotton seed*.tw.
17. soybean oil/
18. intralipid.tw.
19. nutrilipid.tw.
20. ((soy bean or soybean) adj4 (oil* or fat* or sauce*)).tw.
21. (so?a adj4 oil*).tw.
22. so?aoil*.tw.
23. (soy adj4 oil*).tw.
24. soyacal.tw.
25. travamulsion.tw.
26. (sunflower adj4 oil*).tw.
27. helianth*.tw.
28. Safflower Oil/
29. (safflower adj4 oil*).tw.
30. liposyn.tw.
31. (grapeseed adj4 oil*).tw.
32. or/1-31
33. exp Cardiovascular Diseases/

34. cardio*.tw.
35. cardia*.tw.
36. heart*.tw.
37. coronary*.tw.
38. angina*.tw.
39. ventric*.tw.
40. myocard*.tw.
41. pericard*.tw.
42. isch?em*.tw.
43. emboli*.tw.
44. arrhythmi*.tw.
45. thrombo*.tw.
46. atrial fibrillat*.tw.
47. tachycardi*.tw.
48. endocardi*.tw.
49. (sick adj sinus).tw.
50. exp Stroke/
51. (stroke or stokes).tw.
52. cerebrovasc*.tw.
53. cerebral vascular.tw.
54. apoplexy.tw.
55. (brain adj2 accident*).tw.
56. ((brain* or cerebral or lacunar) adj2 infarct*).tw.
57. exp Hypertension/
58. hypertensi*.tw.
59. peripheral arter* disease*.tw.
60. ((high or increased or elevated) adj2 blood pressure).tw.
61. exp Hyperlipidemias/
62. hyperlipid*.tw.
63. hyperlip?emia*.tw.
64. hypercholesterol*.tw.
65. hypercholester?emia*.tw.
66. hyperlipoprotein?emia*.tw.
67. hypertriglycerid?emia*.tw.
68. exp Arteriosclerosis/
69. exp Cholesterol/
70. cholesterol.tw.
71. “coronary risk factor* ”.tw.
72. Blood Pressure/
73. blood pressure.tw.
74. or/33-73
75. 32 and 74
76. randomized controlled trial.pt.
77. controlled clinical trial.pt.
78. randomized.ab.
79. placebo.ab.
80. drug therapy.fs.
81. randomly.ab.
82. trial.ab.
83. groups.ab.
84. 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83
85. exp animals/ not humans.sh.
86. 84 not 85

87. 75 and 86

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