PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Dietary fatty acids in the secondary prevention of coronary heart disease: a systematic review, meta-analysis and meta-regression
AUTHORS	Schwingshackl, Lukas; Hoffmann, Georg

VERSION 1 - REVIEW

REVIEWER	Darisuh Mozaffarian Harvard University, USA Dr. Mozaffarian reports ad hoc travel reimbursement or honoraria from Bunge, Pollock Institute, Quaker Oats, and Life Sciences Research Organization; ad hoc consulting fees from Foodminds, Nutrition Impact, Amarin, Astra Zeneca, and Winston and Strawn LLP; membership, Unilever North America Scientific Advisory Board; and chapter royalties from UpToDate.
REVIEW RETURNED	02-Jan-2014

GENERAL COMMENTS	Major Comments: 1. The authors have done much work to attempt to elucidate an important scientific question. Unfortunately, several fundamental problems exist in their methodologic and analytic approach. As such, their current findings are not interpretable. I sincerely hope they will revise their work in order to supply relevant, appropriate findings to the literature, rather than contributing to further confusion with an invalid analysis. 2. The largest problem is the combining, interpreting, and presenting of these very different dietary interventions as a test of “low SFA” vs. high SFA”. For example, de Lorgeril was primarily an ALA trial, not a SFA-reduction trial; the WHI reduced SFA but also reduced PUFA and MUFA; and so on. Given the very different combinations of both intervention and control dietary fatty acids in each trial, it is wholly inappropriate to combine and interpret these simply as a test of SFA change. The visually pleasing but methodologically incorrect Forrest plots, and their underlying crude analyses, must be deleted. The correct approach is multivariable (not unadjusted, as done by the authors) meta-regression, in which all dietary fatty acid changes are included together in a meta-regression model. This will provide a valid approach to pooling the overall summary evidence from these trials of effects of these dietary fats on CVD in secondary prevention. In these multivariable meta-regressions should be included key trial characteristics and changes in SFA, PUFA, MUFA, and protein as percent energy (i.e., modeling replacement with carbohydrate). A second multivariable meta-regression can replace total PUFA with separate values for ALA and LA. 3. Missing exposure data: For many of the trials, values are missing

	for % energy from various fatty acids, including for control groups and also for LA and ALA in both groups. For control groups, these values should be imputed based on average background dietary intakes in similar populations at that time period. For evaluating LA, all of the trials that reported on total PUFA should be included. None of these trials except Lyon had a major focus on n-3, and as such, total PUFA in each of these other trials would be nearly all (90%+) LA. In addition, nearly all these trials reported the type of vegetable oil used, so that the proportion of LA and ALA in total PUFA can be directly calculated. 4. Multiple outcomes: The authors evaluated 5 clinical outcomes, and only found a borderline significant result (P=0.02) for one of these. I do not favor Bonferroni correction for meta-analyses with multiple outcomes, but at a minimum all null findings should be given equal weight in the presentation of results in the Abstract, Figures, Results text, and Discussion. 5. Heterogeneity appears substantial. Sensitivity analyses should evaluate the influence of single trials on each meta-regression result. 6. Other RCT data from the Singh group has been questioned for veracity and withdrawn by the publishing journal. As such, this trial should be dropped, or included only in sensitivity analyses. 7. The blood lipid outcomes (TC, HDL, LDL, etc.) should be dropped from the meta-analysis. Other published meta-analyses, including far more studies, have established the well-documented effects of dietary fats on blood lipids, and these results add nothing but publication bias and missing data riddled confusion. 8. Introduction: The state of current knowledge should be more clearly presented, in particular the strong evidence that effects of change in SFA cannot be considered independent of the specific replacement nutrient. Exchanging SFA for PUFA, MUFA, carb, or protein (or different subsets of foods within these nutrients) would logically, and based on all available evidence, have very different effects on CVD risk factors and CVD events. This is the crux of the issue, and should be clearly presented and described in the Intro, with appropriate citations. For example, the review by Skeaff, and the follow-up final report from the FAO, clearly discuss the issue of effect modification by nutrient replacement. The US Dietary Guidelines also discuss this issue. Analyses of 11 pooled cohorts by Jakobsen et al., as well as the investigation by Mozaffarian et al. (citation #6 in the present manuscript) also review this key issue. The Introduction should be accordingly revised. This will also provide the relevant background to explain the need for methodologic rigor in pooling the results of these trials by multivariable meta-regression, with carbohydrate as the replacement.

REVIEWER	Franca Marangoni Nutrition Foundation of Italy
REVIEW RETURNED	04-Jan-2014

GENERAL COMMENTS	It is an interesting systematic review and metanalysis of the available data on the role of saturated fatty acids in the secondary

	prevention of coronary heart disease. While the relationship between saturated fats and prevention seems to be supported by the described data, the associations between other fatty acid classes do not appear to be confirmed. For example the Authors state that Statistically significant dose-response relationships could be detected between PUFA (mixed n6 and n3) intake and changes in TC as well as between absolute intake of saturated fatty acids and risk of allcause mortality (p=0.040). However they find a direct association between intakes of linoleic acid, that represents most of PUFA in the diet, and CVD mortality and CVD events. Due to the low number of data available on different PUFA, and on linoleic in particular, in the selected studies, I suggest to focus on the benefits associated to lower dietary SFA rather than to all the fatty acids. In fact this evaluation would need a different selection of the literature. I suggest to focus the paper on the effects benefits do lower SFA rather than on all the fatty acid classes

REVIEWER	Robin Christensen, MSc, PhD; Senior Biostatistician Musculoskeletal Statistics Unit (MSU) The Parker Institute, Department of Rheumatology. Copenhagen University Hospital, Bispebjerg and Frederiksberg. Dr. Christensen is involved in many health-care initiatives and research that could benefit from wide uptake of this publication (including Cochrane, OMERACT, and the GRADE Working Group). Musculoskeletal Statistics Unit, The Parker Institute is grateful for the financial support received from public and private foundations, companies and private individuals over the years. The Parker Institute is supported by a core grant from the Oak Foundation; The Oak Foundation is a group of philanthropic organizations that, since its establishment in 1983, has given grants to not-for-profit organizations around the world.
REVIEW RETURNED	02-Feb-2014

GENERAL COMMENTS	This is a good paper on highly relevant "Public Health issue". The authors investigate the effects of diets low in SFA vs. diets "more SFA" on all-cause mortality, cardiovascular mortality, cardiovascular events (myocardial infarction, stroke), and cardiovascular risk factors in subjects with established CHD. The authors have done a good job; the meta-analysis approaches are contemporary and state-of the art. I only minor comments to consider: The project would benefit a lot from the authors trying to communicate "the quality of the evidence" (ie, the confidence in the estimates) - as that is what dietary guidelines panels would need. Thus, also given by the current BMJ policy, the authors should discuss whether the evidence is downgraded according to the recommndations from the GRADE Working Group. I would strongly recommend the authors to present their meta-analyses in an "Evidence Profile Table".

	In the abstract the authors should include the observed inconsistency (I-squared measure); and mention whether the heterogeneity would likely downgrade our confidence in the estimates. From a "content expert perspective" I would like to see an ancillary analysis addressing whether the length of the fatty acids change the hazards..... Some argue that e.g. the C18:0 can be considered a protective agent. - Thus, the simple question is, are all SFA's hazardous(?) This is a good paper; definitely worth publishing.

VERSION 1 – AUTHOR RESPONSE

Reviewer Name Darisuh Mozaffarian
Institution and Country Harvard University, USA
Please state any competing interests or state 'None declared': Dr. Mozaffarian reports ad hoc travel reimbursement or honoraria from Bunge, Pollock Institute, Quaker Oats, and Life Sciences Research Organization; ad hoc consulting fees from Foodminds, Nutrition Impact, Amarin, Astra Zeneca, and Winston and Strawn LLP; membership, Unilever North America Scientific Advisory Board; and chapter royalties from UpToDate.

Major Comments:

1. The authors have done much work to attempt to elucidate an important scientific question. Unfortunately, several fundamental problems exist in their methodologic and analytic approach. As such, their current findings are not interpretable. I sincerely hope they will revise their work in order to supply relevant, appropriate findings to the literature, rather than contributing to further confusion with an invalid analysis.

2. The largest problem is the combining, interpreting, and presenting of these very different dietary interventions as a test of "low SFA" vs. high SFA". For example, de Lorgeril was primarily an ALA trial, not a SFA-reduction trial; the WHI reduced SFA but also reduced PUFA and MUFA; and so on. Given the very different combinations of both intervention and control dietary fatty acids in each trial, it is wholly inappropriate to combine and interpret these simply as a test of SFA change. The visually pleasing but methodologically incorrect Forrest plots, and their underlying crude analyses, must be deleted. The correct approach is multivariable (not unadjusted, as done by the authors) meta-regression, in which all dietary fatty acid changes are included together in a meta-regression model. This will provide a valid approach to pooling the overall summary evidence from these trials of effects of these dietary fats on CVD in secondary prevention. In these multivariable meta-regressions should be included key trial characteristics and changes in SFA, PUFA, MUFA, and protein as percent energy (i.e., modeling replacement with carbohydrate). A second multivariable meta-regression can replace total PUFA with separate values for ALA and LA.

COMMENT: According to the reviewer´s suggestions, we revised the analyses of the different interventions trials, previously presented as low vs. high SFA. Dietary interventions are now differentiated as reduced/modified fat diets according to approaches chosen by other meta-analyses as well (e.g. Hooper et al. 2012, Skeaff and Miller, 2009). Consequently, literature search has been updated (now including the CVD mortality and CVD event data from the trial by Ramsden et al., 2013).
As requested by the referee, a multivariate meta-regression was performed including all dietary fatty

acids together with a second multivariate meta-regression including LA and ALA values. Due to the reduced degrees of freedom of the main analysis, we decided that performing both univariate and multivariate meta-regression analyses was appropriate.
Since only a low number of studies provided data on protein, the respective potential effects of the macronutrient (available only for 5 trials) could not be estimated.
Likewise, modelling replacement with carbohydrates was not possible, although previous meta-analyses of cohort studies implemented this procedure. However, in the meta-analysis by Skeaff and Miller (2009), RCTs were classified into 4 categories: (1) diets involving a change in the PUFA:SFA ratio, (2) diets involving a reduction in total fat, (3) diets involving an increase in fish or fish oil intake, (4) diets involving an increase in foods rich in α-linolenic acid. In the meta-analysis of RCTs by Hooper et al. (2012), 3 subgroups were combined in the main analysis: (1) low fat diets, (2) modified fat diets, (3) and combination of (1) + (2). Thus, since previous meta-analyses synthesized these different dietary approaches into one analysis, we assume that our new analysis is valid.
In addition, we performed sensitivity analyses for PUFA vs. SFA trials focusing on secondary prevention and sensitivity analysis for trials adopting “fish advice” vs. “no fish advice”.
In the Discussion section of the revised manuscript, the limitations of findings of the meta-regressions analyses performed in the present study are clearly stated, i.e. that the results must be carefully interpreted and used only to generate hypotheses.

3. Missing exposure data: For many of the trials, values are missing for % energy from various fatty acids, including for control groups and also for LA and ALA in both groups. For control groups, these values should be imputed based on average background dietary intakes in similar populations at that time period. For evaluating LA, all of the trials that reported on total PUFA should be included. None of these trials except Lyon had a major focus on n-3, and as such, total PUFA in each of these other trials would be nearly all (90%+) LA. In addition, nearly all these trials reported the type of vegetable oil used, so that the proportion of LA and ALA in total PUFA can be directly calculated.

COMMENT: In agreement with the Reviewer´s comments, we imputed the values for dietary fatty acids for three study control groups (Ball et al. 1965, Leren et al. 1968, and Rose et al. 1965) on average background dietary intakes in similar populations at that time period, the corresponding data were derived from the Diet Heart Study control group (1968). Values are given in the revised version of Table 1. In addition, as suggested by the Referee, LA was calculated from PUFA content (as 90% of PUFA content). Information on the vegetable oil provided for the interventions was given by five study groups, so that LA or ALA content could be calculated.

4. Multiple outcomes: The authors evaluated 5 clinical outcomes, and only found a borderline significant result (P=0.02) for one of these. I do not favor Bonferroni correction for meta-analyses with multiple outcomes, but at a minimum all null findings should be given equal weight in the presentation of results in the Abstract, Figures, Results text, and Discussion.

COMMENT: In the revised manuscript, only 4 clinical outcomes were included (it was not possible to perform meta-regression for stroke, since only 4 data points were available). The findings of the new analyses were given equal weight within the corresponding sections.

5. Heterogeneity appears substantial. Sensitivity analyses should evaluate the influence of single trials on each meta-regression result.

COMMENT: As shown in the revised versions of Table 2 and 3, respectively, heterogeneity was substantial. As requested by the Referee, the influence of single trials on the meta-regression results was evaluated.

6. Other RCT data from the Singh group has been questioned for veracity and withdrawn by the

publishing journal. As such, this trial should be dropped, or included only in sensitivity analyses.

COMMENT: The study by Singh et al. was excluded from the main analysis, but included in the sensitivity analyses.

7. The blood lipid outcomes (TC, HDL, LDL, etc.) should be dropped from the meta-analysis. Other published meta-analyses, including far more studies, have established the well-documented effects of dietary fats on blood lipids, and these results add nothing but publication bias and missing data riddled confusion.

COMMENT: The blood lipid outcomes were deleted from the revised version of the manuscript.

8. Introduction: The state of current knowledge should be more clearly presented, in particular the strong evidence that effects of change in SFA cannot be considered independent of the specific replacement nutrient. Exchanging SFA for PUFA, MUFA, carb, or protein (or different subsets of foods within these nutrients) would logically, and based on all available evidence, have very different effects on CVD risk factors and CVD events. This is the crux of the issue, and should be clearly presented and described in the Intro, with appropriate citations. For example, the review by Skeaff, and the follow-up final report from the FAO, clearly discuss the issue of effect modification by nutrient replacement. The US Dietary Guidelines also discuss this issue. Analyses of 11 pooled cohorts by Jakobsen et al., as well as the investigation by Mozaffarian et al. (citation #6 in the present manuscript) also review this key issue. The Introduction should be accordingly revised. This will also provide the relevant background to explain the need for methodologic rigor in pooling the results of these trials by multivariable meta-regression, with carbohydrate as the replacement.

COMMENT: In keeping with the Reviewer´s recommendations, the following paragraph (regarding replacement of SFA for PUFA, MUFA, carb or protein) has been added to the Introduction section of the revised manuscript:
“Exchanging SFA for polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), carbohydrates (CHO), or protein exerted different effects on CVD risk factors and CVD events [5]. In a systematic review and meta-analysis of cohort studies and randomized controlled trials, Skeaff and Miller [6] concluded that there is convincing evidence that replacement of PUFA by SFA decreases the risk of fatal CHD and CHD events, however they could not confirm the hypothesis of a direct association between SFA intake and CHD death [6]. Furthermore, the authors inferred that replacing SFA with CHO had no relation to CHD. The follow-up final report from the FAO stated that SFA intake should not be higher than 10% of total energy consumption and that SFA should be replaced with PUFA [7]. In their meta-analysis of cohort studies, Jakobsen et al. [8] observed that replacing SFA with PUFA reduced the risk of coronary events by 13% and the risk of coronary deaths by 26%, respectively. In contrast, replacement of SFA by CHO or MUFA marginally increased the risk of coronary events, whereas no significant effects on coronary death could be observed.”

Reviewer Name Franca Marangoni
Institution and Country Nutrition Foundation of Italy, Italy
Please state any competing interests or state ‘None declared’: Nutrition
Fatty acids
Lipid metabolism
Cardiovascular prevention

It is an interesting systematic review and metanalysis of the available data on the role of saturated fatty acids in the secondary prevention of coronary heart disease. While the relationship between

saturated fats and prevention seems to be supported by the described data, the associations between other fatty acid classes do not appear to be confirmed. For example the Authors state that Statistically significant dose-response relationships could be detected between PUFA (mixed n6 and n3) intake and changes in TC as well as between absolute intake of saturated fatty acids and risk of allcause mortality (p=0.040). However they find a direct association between intakes of linoleic acid, that represents most of PUFA in the diet, and CVD mortality and CVD events. Due to the low number of data available on different PUFA, and on linoleic in particular, in the selected studies, I suggest to focus on the benefits associated to lower dietary SFA rather than to all the fatty acids. In fact this evaluation would need a different selection of the literature.

I suggest to focus the paper on the effects benefits do lower SFA rather than on all the fatty acid classes

COMMENT: Since Reviewer 1 suggested to keep the focus on all dietary acids, univariate and multivariate meta-regressions were performed. We re-screened the databases with new keywords and a new search strategy and re-searched all potential relevant systematic reviews and meta-analyses. However, no further relevant studies could be identified. Moreover, several additional sensitivity analyses had to be performed (PUFA vs. SFA, fish vs. no fish advice). Thus, it was necessary to adhere to the inclusion of all dietary acids. (LA contents of the intervention and control groups were calculated according to the suggestions of Referee 1). Furthermore, the limitations of findings of the meta-regressions analyses performed in the present study are clearly stated in the Discussion section of the revised manuscript, i.e. that the results must be carefully interpreted and used only to generate hypotheses.

The corresponding points of criticism of Reviewer 1 as well as our comments are as follows:
2. The largest problem is the combining, interpreting, and presenting of these very different dietary interventions as a test of “low SFA” vs. high SFA”. For example, de Lorgeril was primarily an ALA trial, not a SFA-reduction trial; the WHI reduced SFA but also reduced PUFA and MUFA; and so on. Given the very different combinations of both intervention and control dietary fatty acids in each trial, it is wholly inappropriate to combine and interpret these simply as a test of SFA change. The visually pleasing but methodologically incorrect Forrest plots, and their underlying crude analyses, must be deleted. The correct approach is multivariable (not unadjusted, as done by the authors) meta-regression, in which all dietary fatty acid changes are included together in a meta-regression model. This will provide a valid approach to pooling the overall summary evidence from these trials of effects of these dietary fats on CVD in secondary prevention. In these multivariable meta-regressions should be included key trial characteristics and changes in SFA, PUFA, MUFA, and protein as percent energy (i.e., modeling replacement with carbohydrate). A second multivariable meta-regression can replace total PUFA with separate values for ALA and LA.

Own comment: According to the reviewer´s suggestions, we revised the analyses of the different interventions trials, previously presented as low vs. high SFA. Dietary interventions are now differentiated as reduced/modified fat diets according to approaches chosen by other meta-analyses as well (e.g. Hooper et al. 2012, Skeaff and Miller, 2009). Consequently, literature search has been updated (now including the CVD mortality and CVD event data from the trial by Ramsden et al., 2013).
As requested by the referee, a multivariate meta-regression was performed including all dietary fatty acids together with a second multivariate meta-regression including LA and ALA values. Due to the reduced degrees of freedom of the main analysis, we decided that performing both univariate and multivariate meta-regression analyses was appropriate.
Since only a low number of studies provided data on protein, the respective potential effects of the macronutrient (available only for 5 trials) could not be estimated.

Likewise, modelling replacement with carbohydrates was not possible, although previous meta-analyses of cohort studies implemented this procedure. However, in the meta-analysis by Skeaff and Miller (2009), RCTs were classified into 4 categories: (1) diets involving a change in the PUFA:SFA ratio, (2) diets involving a reduction in total fat, (3) diets involving an increase in fish or fish oil intake, (4) diets involving an increase in foods rich in α-linolenic acid. In the meta-analysis of RCTs by Hooper et al. (2012), 3 subgroups were combined in the main analysis: (1) low fat diets, (2) modified fat diets, (3) and combination of (1) + (2). Thus, since previous meta-analyses synthesized these different dietary approaches into one analysis, we assume that our new analysis is valid.
In addition, we performed sensitivity analyses for PUFA vs. SFA trials focusing on secondary prevention and sensitivity analysis for trials adopting “fish advice” vs. “no fish advice”.
In the Discussion section of the revised manuscript, the limitations of findings of the meta-regressions analyses performed in the present study are clearly stated, i.e. that the results must be carefully interpreted and used only to generate hypotheses.

3. Missing exposure data: For many of the trials, values are missing for % energy from various fatty acids, including for control groups and also for LA and ALA in both groups. For control groups, these values should be imputed based on average background dietary intakes in similar populations at that time period. For evaluating LA, all of the trials that reported on total PUFA should be included. None of these trials except Lyon had a major focus on n-3, and as such, total PUFA in each of these other trials would be nearly all (90%+) LA. In addition, nearly all these trials reported the type of vegetable oil used, so that the proportion of LA and ALA in total PUFA can be directly calculated.

Own comment: In agreement with the Reviewer´s comments, we imputed the values for dietary fatty acids for three study control groups (Ball et al. 1965, Leren et al. 1968, and Rose et al. 1965) on average background dietary intakes in similar populations at that time period, the corresponding data were derived from the Diet Heart Study control group (1968). Values are given in the revised version of Table 1. In addition, as suggested by the Referee, LA was calculated from PUFA content (as 90% of PUFA content). Information on the vegetable oil provided for the interventions was provided by five study groups, so that LA or ALA content could be calculated.

Reviewer Name Robin Christensen, MSc, PhD; Senior Biostatistician
Institution and Country Musculoskeletal Statistics Unit (MSU)
The Parker Institute, Department of Rheumatology.
Copenhagen University Hospital, Bispebjerg and Frederiksberg.
Nordre Fasanvej 57
DK-2000 Copenhagen F
Denmark

Please state any competing interests or state ‘None declared’: Dr. Christensen is involved in many health-care initiatives and research that could benefit from wide uptake of this publication (including Cochrane, OMERACT, and the GRADE Working Group).

Musculoskeletal Statistics Unit, The Parker Institute is grateful for the financial support received from public and private foundations, companies and private individuals over the years. The Parker Institute is supported by a core grant from the Oak Foundation; The Oak Foundation is a group of philanthropic organizations that, since its establishment in 1983, has given grants to not-for-profit organizations around the world.

This is a good paper on highly relevant "Public Health issue".
The authors investigate the effects of diets low in SFA vs. diets "more SFA" on all-cause mortality, cardiovascular mortality, cardiovascular events (myocardial infarction, stroke), and cardiovascular risk factors in subjects with established CHD.

The authors have done a good job; the meta-analysis approaches are contemporary and state-of the art.

I only minor comments to consider:

The project would benefit a lot from the authors trying to communicate "the quality of the evidence" (ie, the confidence in the estimates) - as that is what dietary guidelines panels would need.
Thus, also given by the current BMJ policy, the authors should discuss whether the evidence is downgraded according to the recommndations from the GRADE Working Group.

I would strongly recommend the authors to present their meta-analyses in an "Evidence Profile Table".

COMMENT: As requested by the Referee, an evidence profile Table for the main analysis was added. The data was summarized according to similar meta-analyses by Santesso et al. (Eur J Clin Nutr 2012; 66:780-8. doi: 10.1038/ejcn.2012.37) and by Hooper et al. (Cochrane Database Syst Rev 2012;5:CD002137 doi: 10.1002/14651858.CD002137.pub3[published Online First: Epub Date]|).

In the abstract the authors should include the observed inconsistency (I-squared measure); and mention whether the heterogeneity would likely downgrade our confidence in the estimates.

COMMENT: The I-squared measure was added in the abstract, and the likelihood of downgrading the confidence in the estimates by heterogeneity is given in the revised version of Table 2.

From a "content expert perspective" I would like to see an ancillary analysis addressing whether the length of the fatty acids change the hazards..... Some argue that e.g. the C18:0 can be considered a protective agent.

COMMENT: Extraction of chain length was not possible from the available data.

- Thus, the simple question is, are all SFA's hazardous(?)
COMMENT: According to the suggestions of Referee 1, additional analyses have been performed results of which suggest that they are not. However, the limitations of the findings of the meta-regressions analyses performed in the present study should be taken into account, i.e. that the results must be carefully interpreted and used only to generate hypotheses.

This is a good paper; definitely worth publishing.

VERSION 2 – REVIEW

REVIEWER	Dariush Mozaffarian Harvard, USA Dr. Mozaffarian reports ad hoc travel reimbursement or honoraria from Bunge, Pollock Institute, Quaker Oats, and Life Sciences Research Organization; ad hoc consulting fees from Foodminds, Nutrition Impact, Amarin, Astra Zeneca, and Winston and Strawn LLP; membership, Unilever North America Scientific Advisory Board; and chapter royalties from UpToDate.
REVIEW RETURNED	26-Mar-2014

GENERAL COMMENTS	Major Comments:

	1. Several revisions have been made, which have improved the manuscript. A few key questions and revisions remain to be addressed, without which the validity and interpretation of the findings would be problematic. 2. Table 2, Fig S2-S4: Reduced fat and modified fat dietary interventions should not be combined – these are very different interventions. These two types of interventions should be separately pooled; a secondary, sensitivity analyses could pool them all together as presently done. 3. The addition of the meta-regression has improved the manuscript, but the methods for this analysis remain unclear. Assuming that each trial is separately entered into the meta-regression with the dependent variable being the log change in RR in the intervention vs. control, then what independent variables are entered? The appropriate independent variables would be the change in % energy from SFA, MUFA, and PUFA (or LA and ALA) in the intervention vs. control. Yet, from the text, it is not clear that this was done, and it seems possible that the achieved fatty acid intakes in the intervention group (rather than the change in intervention vs. control) were used. The model construction should be clarified; change in intakes should be used; and Table 3 should clarify that each covariate is the change in these fats, in percent energy. 4. The results of the Sydney diet-heart study should be excluded from these analyses. This trial utilized a commercial margarine, known to be high in industrial trans fat, to deliver PUFA to the intervention group. Thus, the results are strongly confounded by high TFA intake. None of the other fat modification trials were known to alter TFA substantially (generally vegetable oils replaced animal fats), and so adjustment for changes in TFA would fail to address this bias. 4. One key fat reduction trial is missing: the WHI. The separate findings were reported in that trial for the subset of women with pre-existing CHD (higher risk with lower total fat) – these findings should be added. 5. To allow interpretation and confirmation of the meta-regression findings, graphs of the univariate meta-regressions should be presented in supplementary figures, i.e. plotting the log-RR vs. the change in % energy in each fat in intervention vs. control, with size of each marker proportional to the weight; and with the name of each specific trial next to the marker. Minor Comments: Intro: The sentence citing the Mensink & Katan meta-analysis should refer to blood lipids and lipoproteins, not “CVD risk factors and CVD events.” Page 16: “RCTs are considered to have a higher grade of quality.” Should add that while this is a widespread belief, RCTs of lifestyle behaviors such as diet are often limited by lack of double blinding, noncompliance, cross-over, and drop-out – as evidenced by the trials in the current meta-analysis – so that well-designed analyses in prospective cohort studies provide important evidence with complementary strengths and limitations.

VERSION 2 – AUTHOR RESPONSE

Major Comments:

Several revisions have been made, which have improved the manuscript. A few key questions and revisions remain to be addressed, without which the validity and interpretation of the findings would be problematic.

1. Table 2, Fig S2-S4: Reduced fat and modified fat dietary interventions should not be combined – these are very different interventions. These two types of interventions should be separately pooled; a secondary, sensitivity analyses could pool them all together as presently done.
COMMENT: According to the reviewer´s suggestions, we removed Table 2 and Fig S2-S5.
The two types of interventions described are now separately pooled (see Fig S2-9 of the revised manuscript), and combined only via sensitivity analysis (see Fig S10-13 of the revised manuscript).

23. The addition of the meta-regression has improved the manuscript, but the methods for this analysis remain unclear. Assuming that each trial is separately entered into the meta-regression with the dependent variable being the log change in RR in the intervention vs. control, then what independent variables are entered? The appropriate independent variables would be the change in % energy from SFA, MUFA, and PUFA (or LA and ALA) in the intervention vs. control. Yet, from the text, it is not clear that this was done, and it seems possible that the achieved fatty acid intakes in the intervention group (rather than the change in intervention vs. control) were used. The model construction should be clarified; change in intakes should be used; and Table 3 should clarify that each covariate is the change in these fats, in percent energy.
COMMENT: As requested by the Referee, the uni- and multivariate meta-regressions were revised, and the methodological steps are now described in the Method section of the revised manuscript:
“A random-effects univariate meta-regression was performed to examine the association between the change in % energy from SFA, PUFA (mixed n-6 and n-3), MUFA, as well as linoleic acid in the interventions vs. control groups, and the dependent variables (log change relative risks for all-cause mortality, CVD mortality, cardiovascular events, and myocardial infarction). Furthermore, multivariate analyses were performed including all dietary fatty acid changes in a meta-regression model.”

In contrast to the previous version of the manuscript, this analysis could not be performed for α-linolenic acid anymore, since now only 3 data-points (% energy change between intervention vs. control) are available.

Table 3 was modified according the Reviewer´s suggestions.

3. The results of the Sydney diet-heart study should be excluded from these analyses. This trial utilized a commercial margarine, known to be high in industrial trans fat, to deliver PUFA to the intervention group. Thus, the results are strongly confounded by high TFA intake. None of the other fat modification trials were known to alter TFA substantially (generally vegetable oils replaced animal fats), and so adjustment for changes in TFA would fail to address this bias.
COMMENT: A sensitivity analysis was performed without the data of the SDHS trial resulting in a confirmation of the primary analysis. Therefore, we did not exclude the study. The sensitivity analysis is now mentioned in the Results section of the revised version of the manuscript.

The Reviewer mentioned this comment not in the previous revision round.
Nevertheless a sensitivity analysis was performed excluding the SDHS, and this not alter the results

of the primary analysis.

Ramsden et al. 2013:“Another factor that could have been altered by the intervention is dietary trans fatty acids, which are known to raise total and low density lipoprotein cholesterol 61 and have been associated with increased cardiovascular risk in observational studies.62 This association was not widely appreciated during the SDHS, and the trans fatty acid content of participants’ diets was not recorded. Restriction of common margarines and shortenings (major sources of trans fatty acids) in the intervention group would be expected to substantially reduce consumption of trans fatty acids compared with the control group. Conversely, some of this reduction in trans fatty acids in the intervention group may have been offset by small amounts of trans fatty acids in the safflower oil polyunsaturated margarine. Although the precise composition of this margarine was not specified, it was selected for the study because of its ability to lower blood cholesterol and its high PUFA to SFA ratio,22 two characteristics of margarines that contain comparatively low amounts of trans fatty acids.63 Because dietary trans fatty acids are predominantly 18-carbon MUFA isomers,64 the recorded changes in MUFAs probably included small amounts of trans fatty acids in both groups. Statistical adjustment for changes in MUFAs (an imperfect surrogate for trans fatty acids) in sensitivity analyses did not noticeably alter the observed relation between LA and increased risk of cardiovascular death in the intervention group (data not shown). Collectively, these observations indicate that changes in trans fatty acid were unlikely to play a substantial role in the findings reported here. Nevertheless, the SDHS dataset does not contain sufficient information to rule out the possibility that changes in nutrients other than n-6 LA and SFAs could have contributed to, or reduced, the observed unfavorable effects of the LA intervention.”

4. One key fat reduction trial is missing: the WHI. The separate findings were reported in that trial for the subset of women with pre-existing CHD (higher risk with lower total fat) – these findings should be added.
COMMENT: The participants of the WHI trial with a CVD history (n=2277, 908 in intervention, 1369 in comparison group, respectively) were already included in the previous versions of the manuscript (Howard BV, Van Horn L, Hsia J, et al. Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA : the journal of the American Medical Association 2006;295(6):655-66).

5. To allow interpretation and confirmation of the meta-regression findings, graphs of the univariate meta-regressions should be presented in supplementary figures, i.e. plotting the log-RR vs. the change in % energy in each fat in intervention vs. control, with size of each marker proportional to the weight; and with the name of each specific trial next to the marker.
COMMENT: According to the Reviewer´s suggestion, the bubble plot graphs (with size of each marker proportional to the weight, and with the name of each specific trial (i.e. first author, et al. when necessary, and year of publication) assigned next to the marker) are now given in the supplemental materials (see Supplemental Fig S14-29 of the revised manuscript).

Minor Comments:

Intro: The sentence citing the Mensink & Katan meta-analysis should refer to blood lipids and lipoproteins, not “CVD risk factors and CVD events.”
COMMENT: The corresponding sentence was corrected.

Page 16: “RCTs are considered to have a higher grade of quality.” Should add that while this is a widespread belief, RCTs of lifestyle behaviors such as diet are often limited by lack of double blinding, noncompliance, cross-over, and drop-out – as evidenced by the trials in the current meta-analysis – so that well-designed analyses in prospective cohort studies provide important evidence with

complementary strengths and limitations.
COMMENT: The corresponding information was added.