REFeree comments

Reviewer: 1

Recommendation:

Comments:
The authors have recovered previously unpublished data from the randomized phase of Minnesota Coronary Survey, called the Minnesota Coronary Experiment, which was a trial substituting linoleic acid (LA) for saturated fatty acids (SFA). The trial was conducted from 1968 to 1973 among 9,423 institutionalized adults. The recovery of this data is a major accomplishment and publication of the results will add to the evidence base regarding the diet-heart hypothesis. However, I have several concerns about the description, analysis, and interpretation of the results as detailed below.

Major concerns:

I did not find a clear description of how the participants were followed for events in the manuscript or in the supplemental material. Given the very large degree of censoring, I would guess that events were only detected while the participants were hospitalized, but this should be stated directly if true. It appears that only 24% of participants were followed for at least a year. This is a limitation both of the initial trial and of the current analysis. The reasons that people were not followed (eg leaving the hospital, dying) do not seem to have been recovered.

We have now directly stated in Methods Section (page 9) that “Participants were only followed while they were inpatients at the study hospitals”.

We have edited the limitations section (page 31) as follows:
“The MCE also had several important limitations in study design and generalizability. Participants were only followed while hospitalized and only about a quarter of randomized participants remained in the study for one year or longer. Although the original investigators emphasized this subsample and believed the longer follow-up to be more informative, it is a limitation that the association between serum cholesterol and death can now only be examined among those who survived the first year and remained hospitalized.”

There was a huge range of observed changes in serum cholesterol. It is not surprising that people with very large decreases in cholesterol had a very high risk of mortality. A decrease of 100 mg/dl would raise concern that there was a serious underlying illness like cancer (or perhaps measurement error) in a person who was not undergoing treatment to reduce cholesterol or had a moderately effective intervention like the diet used in this study. A 20 mg/dl decrease could be associated with lower mortality while a 50 or 100 mg/dl decrease could be associated with higher mortality. For this reason, it is not appropriate to model change in cholesterol as a linear continuous variable without testing for non-linear associations. There is some suggestion of a non-linear association in the “percent dead” graphs in figure 6. The sensitivity analyses (described only in the results, not the methods) adjusting for change in BMI and blood pressure would not account for a non-linear association.

Response
The reviewer brings up good points.
First, the figure 6 graphs (which provide only a simplified visual representation) are included so that the reader can get a feel for the change in serum cholesterol in both groups, with the caveat that the data are based on average change and do not account for exposure time.
To clarify this, we revised page 16 of the manuscript as follows: "Figure 6 provides a simplified visual representation of change in serum cholesterol and death in the intervention group, control group and combined groups; Table 4 provides more advanced statistical analyses, with hazard ratios (HR) for crude, adjusted and sensitivity models."

For the more sophisticated analysis (Table 4), we did assess model fit and the cholesterol variable for non-linear associations and our models are adequate. Still, based on your question, we went back to our survival analysis and examined different ways of looking at the association (i.e., breaking cholesterol change into four categories or using fractional polynomials to transform the cholesterol variable). We found absolutely no reason to believe that any reduction in cholesterol (big or small) could be associated with a benefit compared to no change (or increase) in cholesterol, which is in line with the results presented in table 4.

While the risk of death seems to be greatest among those that had very large reductions in cholesterol, there are relatively few subjects at those extremes (this is illustrated in figure 6). Moreover, even at these extremes, the reduction in cholesterol was closely related to the assigned diet group. In other words, changes in linoleic acid and saturated fat seem to have accounted for a substantial portion of cholesterol lowering, even in the participants with the most pronounced reductions.

Our team considered the possibility that underlying illness or frailty (which is associated with both low cholesterol and death) might confound the relationship between the change in serum cholesterol and risk of death. For this reason, we performed sensitivity analysis accounting for two known/cited indicators of frailty (changes in body weight and blood pressure). Although this sensitivity analysis is imperfect, we believe that it is the best that can be done with the available data. We are hopeful that publication of this manuscript will help to recover the rest of the MCE data, which includes variables that could potentially be used to better adjust for frailty as well as post-mortem assessments.

We would also like to point out that the manuscript is written in a cautious manner, with a major emphasis on study limitations. In response to the reviewer, we have added the additional sentence to the limitations section "Even though we used RCT data, the analysis of the association between serum cholesterol and death is observational in nature. Therefore, it is not possible to examine causality or to disentangle serum cholesterol changes due to diet from other factors.

The autopsy cohort includes only people who died. Cox models are not appropriate for this type of data. As a minor point, the authors call these results incidence rate ratios, but the other results from Cox models are called hazard ratios for unclear reasons.

- We only used Cox models in the autopsy sample for the results in Webtable 7. Cox regressions were used to model the association between serum cholesterol and myocardial infarcts (presence/absence). We presented hazard ratios to take into account differences in person-time, but the findings are essentially the same using odds ratios (i.e., no evidence of an association), which are now shown in webtable 7. We have clarified the analysis section.

- In the manuscript we present an incidence rate ratio for the association between randomization group assignment and myocardial infarcts in the autopsy sample because it is a straight-forward way to compare events accounting for person-time. The IRR as we list in the paper is 1.90 (p=0.04). The risk ratio (not reported) is 1.88 (p-value 0.01).

While I concur with the statement that the RCT evidence does not provide support for an effect of substituting LA for SFA on CHD or all-cause mortality, the meta-analysis does not include non-fatal CHD events. Many of the trials of statins, many of which have a larger impact on cholesterol than the diet intervention, have not shown an effect on mortality, but do show a decrease in CHD events.
We have now added a composite endpoint (MI plus CHD death) and MI alone as exploratory outcomes in our meta-analysis. We were initially split on the decision to include them because there are only five RCTs that provided vegetable oils rich in LA (without added n-3 EPA+DHA) in place of SFA for CHD death and all-cause mortality. One of these five RCTs, the SDHS, did not report non-fatal events, and another (the MCE) reported only deaths and a partial composite endpoint (Acute and Silent Myocardial Infarctions plus Sudden Deaths). This composite endpoint appears to be missing other coded CHD deaths. So this leaves only 3 RCTs that reported non-fatal MIs, making interpretation very difficult. We did attempt to calculate non-fatal MI from the MCE by subtracting deaths attributed to "Cardiac Arrest, Heart Block" (making the assumptions that "Cardiac Arrest, Heart Block" is equivalent to "Sudden cardiac deaths" and that all MIs were non-fatal). Moreover, non-fatal MIs could also be misleading since diet-group assignment could alter risk of death, duration of diet exposure and/or the risk of death after a non-fatal event.

Given the paucity of RCTs and these critical deficiencies, diet-heart meta-analyses reporting non-fatal CHD events or composite endpoints should be interpreted with caution. That said, in response to the reviewer, we performed an exploratory sensitivity analyses of the composite endpoint (MI plus CHD death) and non-fatal MI alone (Web Appendix Part 2, webfigures 11 and 12). Neither provided any indication of benefit.

Figures 8, 9, and 10 do not present data directly relevant to the current study and seem more appropriate to a review of the topic than an original research article.

We agree that this manuscript provides a review of the topic as well as original research. Given the state of the literature on this topic, we believe that each of these figures provides key context needed to understand the history and potential importance of the diet-heart paradigm.

Minor comments:

Abstract: The “association between changes in serum cholesterol and death” is not precisely an outcome.

- We agree with the reviewer that this association is not precisely an outcome, but we wrote it that way to avoid implying that we analyzed between-group differences in mortality.

Table 3 (page 8) is referenced in the text before Table 2 (page 14).

- We attempted to switch the order, but think it would be easier for the reader to follow as is. The reason is that aside from providing information on the intervention and control diets, this table shows key information for the Results section “Did the MCE intervention lower serum cholesterol?” on page 14.

Additional Questions:
Please enter your name: Emily B Levitan
Reviewer: 2

Recommendation:

Comments:
This is the revised version of this manuscript. The (positive and supportive) general comments made about the original manuscript remain. Authors had received a number of comments from two reviewers and from BMJ Editors. In my view authors have responded fully and professionally to these comments and have produced an improved manuscript. Writing style is improved and is more focussed and numerous points are clarified or discussed more fully. I am satisfied with this version of the manuscript which is timely, novel and interesting. My only comment at this stage is whether the meta-analysis component should be published as a full paper outside of the report of findings from MCE. This is a decision for BMJ editors.
Additional Questions:
Please enter your name: Philip Calder