PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Lack of an Association or an Inverse Association Between Low-Density-Lipoprotein Cholesterol and Mortality in the Elderly. A Systematic Review
AUTHORS	Ravnskov, Uffe; Diamond, David; Hama, Rokuro; Hamazaki, Tomohito; Hammarskjöld, Björn; Hynes, Niamh; Kendrick, Malcolm; Langsjoen, Peter; Malhotra, Aseem; Mascitelli, Luca; McCully, Kilmer; Ogushi, Yoichi; Okuyama, Harumi; Rosch, Paul; Schersten, Tore; Sultan, Sherif; Sundberg, Ralf

VERSION 1 - REVIEW

REVIEWER	David H Newman Icahn School of Medicine at Mount Sinai, NY
REVIEW RETURNED	25-Nov-2015

GENERAL COMMENTS	The manuscript submitted tackles a difficult, vexing, and broadly important issue: the association between cholesterol levels and clinical outcomes. For years this issue has been widely misunderstood and, it would seem, generally mis-perceived. Clarity can come only from transparent, well done, comprehensive data analyses of the topic. The authors are to be lauded for addressing this tremendous, and tremendously controversial, topic, as well as for their apparently quite deep literature review. To be frank the results as written are fascinating, the data seem important, and the ripples of such a study would be large. I am tempted by these attributes. In the end, however, I am frustrated to note that in the manuscript's current form the methods are deeply inadequate, making any conclusions from the reported data unusable. To keep this review brief, and of greatest possible use to the intrepid and obviously diligent authors, I would simply refer them to the PRISMA website and checklist (http://www.prisma-statement.org/). This will be, I believe, of greatest utility in attempting to craft a proper study—an appropriate systematic review—of the critical question being asked here. I suggest going through the checklist and starting over with your important effort. I would also suggest finding a researcher with considerable scientific writing experience to help rewrite the introduction and discussion sections. While both sections currently make for interesting reading, neither adheres properly to the common elements necessary for a scientific paper in the peer reviewed journal community (the

	discussion section is, in this regard, more needy than the introduction). As for the Methods and Results sections the authors will see that the PRISMA checklist should help them to reimagine both in a way that will be more satisfactory both to the author group and to any target journal receiving the next submission. Again, I laud the authors for taking on the subject matter and hope to see more from them, with the above caveats, in the future.

REVIEWER	Flávio Danni Fuchs Division of Cardiology, Hospital de Clinicas de Porto Alegre Brazil
REVIEW RETURNED	30-Nov-2015

GENERAL COMMENTS	This contribution has two major merits: it addresses a topic that has not been contemplate by the literature and collects enough data to support the interpretation that LDL-C is not associated with all-cause mortality in the elderly. It is right as well to provide some explanations for the absence of association and to conclude that we should look for something else to explain the risks for mortality in elderly individuals. Unfortunately, this review fails in two major points, the description and use of methods to perform a systematic review and the overall presentation of the manuscript. The English is grammatically correct but the scientific style is far from the usual. The authors should discuss their own findings and leave aside at least the detailed discussion about several explanations for the absence of association. The tone is excessively opinionated.

VERSION 1 – AUTHOR RESPONSE

We appreciate the positive response both reviewers provided for our manuscript. We have taken into account all of their comments with the outcome being that the revised manuscript is a significant improvement over the initial submission.

We agree that our paper included too many unnecessary and controversial issues. In the new version we have shortened and simplified the discussion and described our methods in more detail, and any discussion, which may have been viewed as opinionated, has been removed.

As mentioned by the reviewers it is an important issue. In the discussion we have therefore focused on the perceived view of the role of cholesterol in heart disease and provided a literature-based explanation for why the large majority of the studies have shown that a high level of LDL-C is either beneficial or neutral as regards total and cardiovascular mortality in elderly people.

We have also followed the PRISMA statement. We have not attached a flow diagram because we did not find it necessary to inform readers about the number of studies where the authors only had analysed total cholesterol, or the number of studies where the association between LDL-C and mortality was not reported, or the number of papers published in other languages, as this information would not be relevant to the outcome of the analysis.

VERSION 2 – REVIEW

REVIEWER	David Newman Independent (most recent institution Mount Sinai School of Medicine, NY), USA
REVIEW RETURNED	03-Mar-2016

GENERAL COMMENTS	Once again appreciate this effort to offer a novel review of a critically important, largely overlooked topic (true vs perceived and assumed associations between serum cholesterol and mortality). In my comments here I concentrate primarily on the issue of validity of the review and its results, ie the Methods section (and, related, the authors' handling of the PRISMA checklist). While the manuscript is in many ways exciting and interesting the current Methods section is inadequate for readers to assess whether the results as reported are a valid representation of the collected works of the included studies, or a valid answer to the question the review asks. The methods section in a high quality systematic review tends to be as long as or longer than the results, partly because of the many PRISMA and MOOSE methodologic elements that must be addressed. I would suggest finding an example of a high quality review and examining the Methods section for comparison, and potentially as a model. Specifically, there are PRISMA/MOOSE elements of a SR that have larger and smaller potential impacts on validity. In particular, elements that are designed to reduce bias in the selection of studies, in data reporting, in data extraction from index studies, and in the critical appraisal and analysis of index studies are typically the most powerful elements. It follows that these are also the most indispensable if investigators hope for their work to be noticed, disseminated, and ultimately to affect thinking more broadly. This manuscript is reaching to have such an impact, and in its best iteration will have such an impact. The details that affect validity will therefore be highly scrutinized by many bright minds in the typical post-publication review process, and this should be both anticipated and prepared for. In the Methods section here the authors do a nice job of describing search terms. My use of these terms in Pubmed yields a similar result. However they choose to query only Pubmed. Not examining alternate databases or non-English language studies is a weakness, as the nature of 'systematic' reviews is by definition to seek all relevant evidence on a topic. This should be addressed in the limitations section, or better yet neutralized by additional searches. It is quite possible additional relevant studies will be found. In addition, the authors exclude studies "with irrelevant issues." It's hard to know what this means, and this kind of vague language will play poorly when critics with an axe to grind examine the paper. There is no flow chart description of study selections and exclusions (the authors' statement that this "would not be relevant to the outcome of the analysis" is inadequate; this assertion would ostensibly always be true for any such flowchart but of course there is a reason that PRISMA includes this element—it is widely accepted to be quite relevant to the interpretation of the outcome of the analysis, to the reproducibility of the review overall, and thus to

	the scientific validity of the work). There is no description of how data were extracted from each study (were data forms used, were abstractors trained, was this process standardized, were terms pre-defined, was there a consensus approach or was it done by one investigator, was there a spreadsheet or a program utilized, how were data pooled, etc.—again, all of these elements help readers and future investigators to understand how much potential bias was neutralized by this process, and how to reproduce the review itself). There is no real analysis of weaknesses or biases from the index studies. The statements in the checklist that the authors have found no evidence of bias in any of the studies is perplexing. There is, naturally, no study without bias. All studies make methodologic choices that minimize some forms of bias and increase other forms. Rare, methodologically pristine studies take all available precautions however this is both exceedingly uncommon, and always in the context of inherent limitations. Cohort studies, which form the core of this review, in particular are prone to many forms of bias related to criterion standards, definitional choices, reporting processes (e.g. survey vs clinical monitoring vs registry databases vs other), adjustment methods, analytic options, etc. There truly is no such animal as a study without biases. It might be worth examining the charts used in most Cochrane reviews for quality assessment. It may also be useful to examine the MOOSE statement on reporting of systematic reviews of observational studies, which similarly notes the importance of quality assessment (preferably by blinded assessors). This is not to say that the purpose of seeking these limitations is to criticize or bash the studies, but it is crucial to the process of extracting, understanding, and making inferences from the data. As has been famously said about meta-analysis: "garbage in, garbage out." Readers must have some sense of the weaknesses in the original data or their perceptions will be skewed favorably and their inferences will reflect an undue certainty about the summary estimates reported. In this case the misperception that would result would be ironic, for the authors quite rightly are attempting to correct a longstanding over-certainty and misunderstanding about the dangers and biological meaning of elevated serum cholesterol. The larger cholesterol misunderstanding is a product of mistakes made over decades as investigators with good intentions presented partial data from improper, biased, and highly selected sources without rigorous or controlled methods, without systematic reporting, and without considering the faulty nature of the original information. It would be unfortunate, and potentially damaging in the long run, to attempt to correct this misunderstanding with anything other than an unimpeachable, methodologically very careful approach to the question.

REVIEWER	Flávio Danni Fuchs Hospital de Clinicas de Porto Alegre, UFRGS, Brazil
REVIEW RETURNED	19-Feb-2016

GENERAL COMMENTS	The authors correct the main points that I have raised before. The "stress" theory as an explanation for the paradoxical association is naïve, since stress itself has been criticized as a risk factor for CVD, but let the readers evaluate this point.

VERSION 2 – AUTHOR RESPONSE

We are gratified that both reviewers were so positive in their remarks about our manuscript.

Professor Fuchs suggests that our observation of a link of stress to elevated cholesterol to heart disease is naïve. We recognize that we have not provided sufficient substantiation of this hypothesis. We have therefore enhanced our coverage of the topic by including references to a meta-analysis and a review about this issue published in BMJ and JACC , respectively (ref. 33 and 34), both of which demonstrated that psychosocial factors strongly influence the course of CHD

Our impression is that Dr. Newman´s primary concern is that we should substantiate our description of the methods and the processes by which we selected the relevant studies. We have therefore included a section with more detailed information on this point.

As English is recognized as the universal language of science, and most medical journals with high impact are in English, we excluded papers published in non-English languages. A few of them might be relevant, but they were published in Chinese or Japanese. Furthermore, by rereading the studies included in our analysis, we found that we have misunderstood the figures in the paper of Tikhonoff et al. These authors reported in fact an inverse association between LDL-C and mortality; or more correct, in three cohorts the associations were mirror-J shaped; in one of them almost U-shaped, but with the lowest risk in the highest quartile. Consequently the results in all of the studies reviewed went in the same direction. We therefore think that it is very unlikely that we should find a study with the opposite result, and even if so, it cannot change our conclusion.

By rereading the studies we also found a few minor errors without importance for the final result. We have marked these errors with yellow as well.

Dr. Newman pointed out that there is no real analysis of weaknesses or biases from the chosen studies. He is right of course. Our approach was to impose minimal bias on our part and in our assessment of the literature by following objective criteria for inclusion/exclusion of studies in our review, without imposing restrictive criteria based solely on whether they appear biased. However, we have added a disclaimer in the limitations section about possible bias.

We have included a flow diagram. However, it is not possible to separate the rejected papers in more detail because most of them were excluded by several reasons.

In summary, we appreciate the support and constructive criticisms both reviewers have provided, which has resulted in a significantly improved manuscript. We hope the editor finds our revised manuscript suitable for publication in BMJ Open because as stated by Dr. Newman, ”the larger cholesterol misunderstanding is a product of mistakes made over decades as investigators with good intentions presented partial data from improper, biased, and highly selected sources without rigorous or controlled methods, without systematic reporting, and without considering the faulty nature of the original information.”

VERSION 3 - REVIEW

REVIEWER	David Newman Most recent institution (no current affiliation): Mount Sinai School of Medicine, NY, USA
REVIEW RETURNED	11-Apr-2016

GENERAL COMMENTS	The authors offer a revision from their prior version of "The Association Between Low-Density Lipoprotein Cholesterol and Mortality in the Elderly. A systematic review". Again, to reiterate, I find the topic important, and the main thrust of the effort both correct and eye opening. My remaining concerns are with methodology and tone. Final decisions about what should be considered acceptable and publishable are journal-specific and therefore above my pay grade. I offer a few comments, none of which is intended to suggest that the paper either is or is not suitable for publication in BMJ Open. These are intended only to reflect remaining sources of potential improvement for the manuscript. -By 2009 PRISMA checklist standards the manuscript is missing most elements (5, 9, 10, 11, 12, 14, 15, 16, 17 (partial), 19, 20, 21, 22, 23, 24, 25 (partial)). Many SR's are missing PRISMA elements, editorial tolerance for this is obviously based on context, importance of the missing elements, and journal. -One missing aspect is an assessment of the bias and potential inference problems in each of the individual studies that constitute the data set being reported here, and a mention of how that might affect the reader's inferences about the data as a whole. The only bias issue that is addressed (three times) seems to be that of serious illness as a source for mortality increases among those with low cholesterol. This is minor, deserves, a single mention, and could be followed with a discussion of many other anticipated and discovered potential sources of bias. -The discussion section gives the impression of being driven by an agenda. There is a difficult and fine line to walk with studies that challenge accepted dogma. I fear that the tone of the discussion section may make readers wonder whether the approach taken to these data was scientific and objective. Could be rewritten in a more objective tone. -Would suggest rewriting the discussion section, taking pains to discuss only issues the data from this review can appropriately shed light on. There is, for instance, no inference that can be made here about people younger than 60. In that vein, would consider removing last paragraph before Discussion section, which seems to be a digression without explanation or methodology. Another example: mentioning the authors' theory that pleiotropic effects of statins explain their impact is reasonable as a potential footnote, but making any kind of forceful or assertive argument about this theory doesn't seem to flow from the data in the review itself.

VERSION 3 – AUTHOR RESPONSE

We appreciate Dr. Newman´s great efforts to improve our paper. We have provided additional information in our response, which addresses all of his concerns

In the start Dr. Newman claims that we have missed most elements of the PRISMA checklist. Obviously I have included the wrong list by mistake, because we have followed all the relevant elements and also added the page numbers where the information is available.

Dr Newman also noticed that the only bias we have addressed is serious illness as a source for mortality increase among those with low cholesterol. However, in the previous manuscript we had included four types of bias, and in the present we have added yet another (see page 3 and pages 8-10).

As suggested by Dr. Newman we have simplified the discussion by excluding the section about LDL-C in younger individuals and we have removed the discussion of pleiotropic effects of statin treatment. We have also changed the overall tone of the paper in an attemp to provide an unbiased scientific and objective assessment of the literature.

We have made a minor change of the paper. When we checked the references, we saw that some of the co-authors appeared both in the study by Fried et al. and in the study by Psaty et al. By rereading these papers it appeared that they have examined the same individuals; the first one after a follow-up of 4.8 years; the other one after 7.5 years. As Psaty et al. has included individuals whose data were incomplete, we have only included the paper by Fried et al.