Dear Dr. Nordestgaard,

Thank you for sending us your paper. We sent it for external peer review and discussed it at our manuscript committee meeting. We recognise its potential importance and relevance to general medical readers, but I am afraid that we have not yet been able to reach a final decision on it because several important aspects of the work still need clarifying.

We hope very much that you will be willing and able to revise your paper as explained below in the report from the manuscript meeting, so that we will be in a better position to understand your study and decide whether the BMJ is the right journal for it. We are looking forward to reading the revised version and, we hope, reaching a decision.

Please remember that the author list and order were finalised upon initial submission, and reviewers and editors judged the paper in light of this information, particularly regarding any competing interests. If authors are later added to a paper this process is subverted. In that case, we reserve the right to rescind any previous decision or return the paper to the review process. Please also remember that we reserve the right to require formation of an authorship group when there are a large number of authors.

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**Report from The BMJ’s manuscript committee meeting**

These comments are an attempt to summarise the discussions at the manuscript meeting. They are not an exact transcript.

Members of the committee were: Wim Weber (Chair), Julie Morris (Statistician), John Fletcher, Elizabeth Loder, David Ludwig, Joseph Ross, Tiago Villanueva.

Decision: Put points

Detailed comments from the meeting:
We thought your study addresses an important and interesting research question. After discussion, however, we felt that it did not add enough to take it further towards publication in The BMJ. We had the following concerns.

The use of cubic spline curves and predefined LDL-C categories with Cox proportional hazards regression to assess the nadir of the U-shaped curve between LDL-C and mortality is a suitable analysis. However, the simple adjustment for regression dilution bias does not seem entirely appropriate in this instance (also see remarks by Freedman). Perhaps results could be presented with and without this adjustment?

It would be helpful to see the inter-relationship between statin treatment and LDL-levels and the association with mortality explored in greater detail. Differences in the position of the nadir and associations with mortality are reported to have been found, although there is no mention of this in the Abstract. Was a formal statistical assessment of a significant interaction made?

Given the much smaller subgroup of patients taking statins, mortality estimates would be expected to have much wider confidence intervals attached, and thus the interpretation of non-significant findings, in particular when making indirect comparisons between subgroups, need to take this into account. Eg. eFigure 6 shows cardiovascular mortality by overall population, no lipid-lowering therapy and lipid lowering therapy. In the text, the researchers state that, "In individuals not receiving lipid -lowering therapy, low LDL-C levels were associated with an increased risk of cardiovascular mortality..." , but, "...no association was found..." for those receiving lipid-lowering therapy. However, the left side of the 'U-shape' for statin-users shows a (non-significant) rise and thus I doubt whether there is a statistically significant difference between the subgroups for low LDL-levels.

It also seems odd that the two subgroups show a rise in cardiovascular mortality for low LDL-levels, but there is no apparent rise for the whole study group.

A number of sensitivity analyses were carried out, and each one showed similar results to the main analysis, but with the association slightly attenuated. It would be interesting to see whether the results remain the same if one analysis incorporating all the 'sensitivity changes' was carried out.

First, please revise your paper to respond to all of the comments by the reviewers. Their reports are available at the end of this letter, below.

In your response please provide, point by point, your replies to the comments made by the reviewers and the editors, explaining how you have dealt with them in the paper.

Comments from Reviewers

Reviewer: 1

Recommendation:

Comments:
1 The paper contains important information, focusing on mortality risk at all levels of LDL-C levels, but I have some concerns about the analyses.

2 The authors largely rely on restricted cubic splines to estimate the LDL-C level associated with the lowest mortality. (This was supplemented by stratified analyses.). Since the focus of the paper is on the LDL-C value associated with the lowest mortality, I'd like to see another modelling technique incorporated in the paper – I'm familiar with 2 other techniques in R: fractional polynomials and P-splines [Govindarajulu et al. 2009], and a quick search indicates that fractional polynomials are
available in Stata. I think that paper would benefit from an additional modeling technique that confirms (or not) the results of the spline models.

3 I’m actually very surprised at the difference in the findings for CVD mortality (U-shaped) and myocardial infarction (monotonic, eFigure 7), and I’m having difficulty in understanding how the 2 associations can differ so much. I’m assuming that MI includes both non-fatal and fatal MI; is this correct? Please provide information on the proportion of CVD deaths due to fatal MIs – I would imagine that fatal MIs make up about 40% of CVD deaths. In the total population there were 2587 MI events and 2428 deaths due to CVD (eFigure 6). Would it be possible for the authors to analyze the relation of LDL-C to CVD mortality excluding fatal MIs – I would think this category would largely represent deaths due to strokes and heart failure; is this correct? Would it be possible to state which category is ‘responsible’ for the association between low LDL-C and CVD death?

4 I realize the constraints of word limits in journal articles, but I think authors could do better than to simply state ‘… provided different results’ (p 3, 32)
The authors adjust their HRs for regression dilution bias, which – in the univariate case – would increase the reported HRs. My understanding, however, is that this type of adjustment, which is appropriate for univariate HRs, may not be appropriate in models that adjust for confounders [Knuiman et al. 1998]. I’d like to see justification for why this approach is appropriate. Further, while adjustment for dilution is appropriate if one if interested in the long-term relationship of LDL-C (which varies over time) to mortality, it may not be appropriate if one is interested in the relationship of an initial LDL-C value to mortality; perhaps this distinction could be clarified as it’s not clear to me if the baseline or long-term association would be of most interest. Also, was the adjustment made by multiplying the regression coefficients by 1/0.64 (p 6 line 32) irrespective of whether a person was followed for 1 year or for 15 years? (Based on the information on p 4, line 11, I’m guessing that the maximum follow-up was about 17 years; is this correct?)

5 A reference for the multiple imputation technique would be helpful (p 6, 40).

6 I’m confused by the adjustment for age (p 6, 54). The authors state that ‘as timescale’; does this mean that the actual age at baseline was used in the regression models or that the time since the LDL-C measurement was used (or both)?

7 The incomprehensible sentence on p 7 (23 to 33) is rather unnecessary as the information is in Figure 2. Please shorten the sentence rather than repeating all the information from a figure..

8 P 8, line 7: what does ‘no association .. on a continuous scale’ mean? Does this mean that the linear component was not statistically significant or than the overall spline was not statistically significant?

9 The interaction in eFigure 5 (which is incorrectly labelled ‘Figure 5’) should be explained. I assume that the p-value is for a term assessing whether the HR differs between, for example, between men and women; is this correct? In addition, was it the linear interaction that was examined or the interaction with all of the splines?

David Freedman, Centers for Disease Control and Prevention

References


Additional Questions:
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Please enter your name: David Freedman
Job Title: Senior Epidemiologist
Institution: Centers for Disease Control and Prevention
Reimbursement for attending a symposium?: No
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Reviewer: 2

Recommendation:

Comments:

In this large Danish cohort, the authors found a U-shaped relationship between LDL cholesterol levels and mortality risk: both low and high LDL-C levels were associated with increased all-cause mortality and the lowest mortality was found at an LDL-C level of 3.6 mmol/L (140 mg/dL). This finding, if confirmed by additional studies, will have important clinical and public health implications.

This finding mimics the "obesity paradox" in terms of BMI and mortality, where low BMI levels are associated with increased mortality and higher BMI levels are also associated with increased risk. However, the obesity paradox is largely explained by methodological issues in the analyses of BMI and mortality, including reverse causation due to preexisting or preclinical diseases and unintentional weight loss, confounding by smoking, etc. It is unclear whether the observed positive association between low LDL levels and mortality also reflects similar methodological problems. In the BMI-mortality literature, when the analyses are confined to healthy middle-aged individuals without chronic diseases, the association between BMI and mortality becomes largely linear.

In the present analysis, the authors did multiple sensitivity analyses to address potential reverse causation by excluding the deaths in the first 5-year of follow-up and individuals with existing chronic diseases such as CVD, cancer, and pulmonary diseases at baseline. However, these exclusions were done sequentially rather than simultaneously. It would be helpful to do these exclusions simultaneously to minimize residual confounding.

Unintentional weight loss due to existing or preclinical chronic diseases has been associated with decreased blood cholesterol and blood pressure. Besides adjustment for BMI levels, it would be important to adjust for the amount of weight loss, if possible.

"Among individuals receiving lipid-lowering therapy, the association between low LDL-C levels and increased risk of all-cause and cancer mortality was much weaker than for individuals not receiving lipid-lowering therapy, while no association was found between low LDL-C levels and cardiovascular or other mortality." This finding needs additional analysis and careful interpretation. Is the interaction between statin treatment and LDL cholesterol significant? Why is low LDL associated with cancer mortality but not CVD mortality among those who were treated with statins? Does this mean that the positive association between low LDL and cancer mortality is not causal? It would desirable for the authors to examine the relationship between LDL levels and the incidence of major cancers in this cohort, if possible.

The authors concluded that "These findings support that use of lipid-lowering therapies should be based on absolute atherosclerotic cardiovascular disease risk rather than on moderately elevated LDL-C levels alone." This is an overstatement given the uncertainty of the causal relationship between low LDL levels and mortality. To a large degree, the clinical guidelines should be based on the evaluation of major clinical events such as MI and CVD incidence rather than total mortality, a complex endpoint that is more susceptible to reverse causation and other biases.

Previous Mendelian Randomization analyses have demonstrated a causal relationship between increased LDL levels and CVD risk. If the authors have genetic data for the cohort participants, it would be worthwhile to conduct a similar MR analysis on mortality outcomes.

Additional Questions:
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