Dear Dr Fišter,

We are writing to appeal the decision on the manuscript BMJ.2016.035582 entitled "Mortality from different causes associated with meat, heme iron, nitrates and nitrites in a cohort of over half a million Americans: The NIH-AARP Diet and Health Study". Below please find the point by point response to the comments made by the reviewers. We also would like to suggest the following reviewers: Dr John D. Potter, Fred Hutchinson Cancer Research Center (jpotter@fhcrc.org); Dr Loic Le Marchand, University of Hawai'i (loic@cc.hawaii.edu); Dr Walter C. Willett, Harvard T.H. Chan School of Public Health (wwillett@hsph.harvard.edu).

Yours sincerely,

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Dear Dr Fister,
We want to thank the BMJ editors and the reviewers for the time they have spent reviewing our manuscript. While we respect the editorial position on the manuscript, we thought it is important to alert you to a problem that you might want to consider for the future. Specifically, we were surprised to receive views, in the form of comments from Dr. Archer, that were not focused on the scientific content of our manuscript, but rather were the repetition of previous claims about the whole field of nutritional epidemiology. These extremely negative views held by Dr Archer against methods commonly and widely used by nutritional epidemiologists have been widely criticized and discussed by numerous well-known scientific colleagues (J Nutr 2015;145:2639–45; Adv Nutr 2015; 6: 230-233; Adv Nutr 2015; 6: 489). We will not repeat these responses here (some of these responses are attached to this email), but wish to point out a few essential elements of the arguments which are relevant to our manuscript:

Reviewer 1
1. While measurement error will always remain a part of observational epidemiology in general, and nutritional epidemiology in particular, the claims that these errors are systematic and lead to bias are not supported by data or evidence.
2. Most of these errors in fact may lead to underestimated effect sizes, and any consistent and sizeable finding is unlikely to be due to measurement error.
3. Most of the claims against the validity of dietary questionnaires stem from energy intake underreporting. Data from validation and biomarker studies show that other self-reported dietary data (except for energy intake) contains low levels of underreporting. Thus, most investigators agree that the energy intake from dietary questionnaires must not be used as the main exposure variable, but is still useful as an adjustment factor for other nutrients.
4. In multiple independent studies, improved diet quality has been shown to be linked to marked reductions in mortality, and it is very unlikely that all these investigators from large studies have made the

same mistake in the same direction.
Nutritional epidemiology has made many important and useful contributions to public health and prevention of many common diseases. However, we do agree that methods used in nutritional epidemiology are far from perfect, and have already discussed some of these limitations in our manuscript, or tried to assess their potential impact by using validation data.
We believe that unless one should wish to use Dr Archer's arguments as a policy guide to reject all manuscripts based on dietary questionnaires (which we strongly disagree with), there is little in terms of specific scientific issues in his comments that yield to reasoning or constructive dialogue. Dr Archer also accuses us of fabrication and falsification based on the fact that he seems to disagree with our methods, which were presented with complete transparency. We think that the choice of a reviewer who is so fundamentally biased against a whole field of research is not useful to inform the Journal's editorial decision. We note that BMJ has recently published or reported on results in 2016 using methods very similar to ours (BMJ 2016;354:i5286; BMJ 2016;354:i5286; BMJ 2016;354:i4586; BMJ 2016;353:i2343; among many others).
Finally, we were disappointed that you would forward a review that claims we may be guilty of scientific misconduct when our methods were transparent and our work is clearly within the mainstream of epidemiologic research. Differences of opinion in scientific studies should be discussed without resorting to potentially libelous statements.
Sincerely,

Reviewer: 2

We would like to than the reviewer for the general comment about the manuscript and its novelty.

Comment by reviewer: *1. The authors have used energy density method as the exposures. Maybe some other methods could also be tried, e.g., residual method.*

> **Response:** We did try using the residual method as well, and the results were identical to those presented. We will add this sensitivity result to the methods and results section.

Comment by reviewer: *2. The exposures have been measured only once at baseline, thus whether the exposures could represent the long-term intake levels remains unclear, particularly if the meat products and intake patterns have changed in the past several decades. Any studies to evaluate the reproducibility and variability of the DHQ in assessing dietary intakes? How about the validity of DHQ in assessing meat intake in the calibration study?*

> **Response:** We agree that the lack of a repeated assessment of diet is a limitation in the NIH-AARP Diet and Health Study, and as we have discussed, this might have biased some of our estimates towards null. We can elaborate more on this issue in the discussion. The reviewer has also suggested using the calibration study to evaluate the DHQ. Such evaluation has already been published (Schatzkin A, et al. Am. J. Epidemiol. (2001) 154 (12): 1119-1125.) showing a correlation of 0.62 in men and 0.70 in women for red meat intake, which was higher than most other intake items tested. We have mentioned these results in our manuscript, and used the data from the calibration study to estimate how much the remaining measurement error can affect our results (Supplementary Table 5). Our findings show that correcting for the measurement error will lead to higher hazard ratios, again confirming the message and conclusion of the manuscript.

Comment by reviewer: *3. The authors have included chicken, turkey, and fish in the same category of white meat, however, it might be better to do a sensitivity analysis to examine the associations separately. Fish and chicken may have different impact on the mortality risk. Some previous literature should also be discussed and compared in the Discussion section.*

Response: We have done separate analyses of fish intake. Fish intake constituted about one third of white meat intake in this population. When we separately analyzed the sources of white meat, the results were similar, but fish intake had weaker associations with all-cause and cause-specific mortality; for example, while the HR for all-cause mortality was 0.93 (95%CI: 0.92-0.94) for each 20 g/1000 Kcal increased daily intake of chicken, the same HR was 0.95 (95%CI: 0.94-0.96) for fish intake. These results are available and can be added to the manuscript, and compared with the literature in the discussion.

Comment by reviewer: *4. In the final model, the authors only included fruit and vegetables intake. Meat intake could be related to other dietary habits, and more fine adjustment of dietary habit could be considered, such as certain dietary patterns (without meat component).*

Response: This is an interesting suggestion; however, adding more dietary variables may lead to bias in the estimates, as the error in these variables are likely to be correlated. We have tried to limit the number of dietary variables in the model, and restrict them to those likely to have an impact on mortality based on previous findings.

Comment by reviewer: *5. In the substitution model, if total meat and red meat were both included in the model, the meaning of red meat was the change in mortality risk by substituting red meat with white meat, while the meaning of total meat in this model was the change in mortality risk by increase of white meat holding red meat constant. Therefore, for white meat, this is the addition model and the results should be comparable to the "addition model" the authors have used. Furthermore, in the substitution model, the increased mortality risk of replacing red meat by white meat should be the reciprocal of the decreased mortality risk of replacing white meat by red meat. Theoretically, this should be the case, and the authors could examine their data and make sure they have used the correct method.*

Response: This is a correct conclusion, and one we have found to be true in our models.

Comment by reviewer: *6. Heme iron can also come from white meat, and the authors can test the relations of heme iron from red meat and heme iron from white meat with the mortality risk. Or the authors can test whether the risk related to red meat was mediated by total heme iron or heme iron from red meat.*

Response: We agree that heme iron may also come from white meat. However, the amount of heme iron from white meat in our study was relatively small (10.3% and 16.0% of total heme iron in men and women, respectively), and models using heme iron from total meat were almost identical to those using only heme iron from red meat. We can add more details about the difference between these two analyses as supplementary material.

Comment by reviewer: *7. Table 1, it is better to show the total number of participants in each quintile of red meat intake. The current table 1 shows the number of deaths in each quintile, and is somewhat confusing.*

Response: We agree and will add this information to Table 1.

Comment by reviewer: *8. Some of the results are unexpected and deserves more discussions. For example, why stronger associations were the mortality from chronic liver diseases and respiratory diseases, rather than cardiovascular disease or cancer? The authors have talked about oxidative stress and other pathways, but those mechanisms are not specific for liver or respiratory system.*

Response: COPD is the main reason for death due to respiratory disease, and might be linked to the formation of reactive nitrogen species, as explained in the discussion. We are ready to add more specific discussion about other associations, when such information is available.

Comment by reviewer: *9. For the mediation analysis, could you examine the proportion of the increased mortality risk associated with processed red meat mediated by nitrate/nitrite and heme iron together?*

Response: This is an interesting idea, however, to the best of our knowledge, the current methods to estimate mediation effect do not allow for a modelling a "combined effect".

Comment by reviewer: *10. In table 2, it will also be better to show the proportions mediated by heme iron for total red meat and unprocessed red meat.*

Response: This is an excellent idea, and we will add it to the manuscript.

Comment by reviewer: *11. The interaction results were not comprehensively discussed in the Discussion section. Are the significant interactions by chance or there are biological rationales for the interactions? How to explain the results?*

Response: As mentioned in the manuscript, we observed lower associations between meat intake and mortality in the presence of another (maybe stronger) risk factor, such as smoking or obesity. We can explain this in more detail in the discussion.

Reviewer: 3

Comment by reviewer: *The response rate at baseline was rather low (~16%) and there is a question of generalizability of the findings. There are two parts to this question. First, estimating incidence or mortality rates is not the focus of the study, therefore the low response rate is not a problem per se.*

Second, because the research hypotheses concern the causal relationships of meat and associated dietary intakes with mortality, distortion of the results due to selection bias will occur if the probability of study participation is associated with the exposure-outcome relationship. The logic is that non-participation at a particular level of meat intake will distort the diet-mortality effect estimate if mortality risk differs between those present in the study and those not present, and further that the probability of participation differs across quintiles of meat intake. This situation may arise despite the prospective study design, as a consequence of the differing propensities of individuals to participate in health research according to their pattern of health behaviour. Although subjects were recruited (and thus diet was measured) many years before disease onset and death, we would need to know something about the life styles and health of those who refused to participate in the study (or at least a subset) before we could exclude selection bias as a partial explanation of the findings.

Response: Non-participation is a major concern in different epidemiologic studies, and as the reviewer has pointed out, it does not always lead to biased estimates. We agree that participants might be more health-oriented than the non-participants, a fact that is true about most similar studies, but there seems no reason to infer that it would hurt the internal validity of the estimates from our study. In fact, only a small number of cohorts large enough to be useful for cancer have accrued representative population samples. To impair external validity, one would have to assume that the non-participants in a study have different exposure-outcome associations than those who did return the questionnaire. Although we do not have detailed information about the non-participants' behaviors or their outcomes, we know that the intake distributions of participants differed from those observed in national surveys (Schatzkin A, et al. Am. J. Epidemiol. (2001) 154 (12): 1119-1125.). They consumed less fat and red meat and more fiber and fruits and vegetables than the general US population in the same age group. But, at the same time, the intake distributions were wider than those in the national surveys. This widening of the intake distributions can somewhat overcome the shift to the left, by including more people in the extreme intake values. The results of our analysis also showed that the associations we observed were fairly comparable between low and high socioeconomic groups. Finally, since its initiation, there have been more than 300 published reports based on the data from the NIH-AARP Diet and Health Study, and many of these findings have been replicated in other cohort studies, and accepted by the scientific community, so we feel it is unlikely that there should be a fundamental bias in all these findings. However, we can certainly include the non-participation rate as one of the limitations of the study, and discuss its potential impact on the findings and conclusions.

Comment by reviewer: *Separately, there is a question about the lack of specificity of the observed health effects of meat intake (see Fig 1). The pattern of risks is remarkably constant across all 10 cause groups, such that red meat intake appears to increase the hazard of death from any cause except Alzheimer's, including infectious causes. This finding suggests that there is some common method error linked with the use of a food frequency questionnaire. For example, the apparently adverse effects of higher red meat intake (and generally protective effects of higher white meat intake) could be the result of reporting bias or what can be called 'behaviour bias'. In other words, it is difficult to distinguish between causal explanation, bias and confounding. Health conscious people have lower mortality risk because they behave well in general, and they may have reported falsely low red meat intake. Other people may*

genuinely have had lower red meat intake because they were health conscious and believed low intake would be salutogenic. Both explanations undermine the claims of causality made in the study, and they should be discussed along with high quality null findings which suggest that a higher red meat diet is not a risk factor for colorectal cancer, one of the health outcomes most firmly linked with red meat intake in popular science. Thus, Spencer et al Cancer Causes and Control 2010 used a prospective case-control design based on food diaries. Ananthakrishnan et al Cancer Epidemiol Biomarkers Prev 2015 in a pooled analysis of 11 studies showed the same association was seen only in retrospective studies, again pointing to the challenges of nutritional epidemiology which are barely mentioned in the manuscript.

Response: The 'behaviour bias' proposed by the reviewer is indeed among the different explanations for many associations observed in epidemiology, and for a lot of important exposures including smoking and obesity. As such, we have tried to avoid direct causality inferences, as most of the evidence in observational epidemiology does not necessarily imply causation by itself. However, independent organizations (WHO International Agency for Research on Cancer (IARC), World Cancer Research Fund, and American Heart Association among them) have found the evidence for the association between red meat and many diseases convincing enough to suggest causality. Our findings show important additional associations that may or may not prove to be causal given the evidence from other reports, or external data from animal or mechanistic studies. But we believe we have an obligation to report these findings, along with different possible explanations and potential limitations. We have tried to explore possible mechanisms by looking at evidence linking the exposures we have studied to oxidative stress, and the role of meat compounds in the process. We think that our result can both provide more evidence as to the nature of meat-disease associations reported previously, and generate new hypotheses to be tested in future studies. Besides, as the reviewer has also pointed out, there are variations across causes of death which are difficult to explain by some big shared confounder alone. We consistently saw no association between meat intake (both red and white meat, along with meat compounds) and Alzheimer's disease; the associations with stroke were much weaker than those with cardiac death (two diseases with many similar risk factors); and finally the associations between both red and white meat intakes with liver disease were consistently stronger than those with other diseases. Death due to infectious diseases, which was the main concern of the reviewer, was among the weakest associations we observed, and is probably affected by changes in the immune system, although we agree that acquiring the infectious agent itself is unlikely to be affected by the amount of meat in the diet.

Finally, we have tried to include different views and important previous literature in the discussion, whether or not they support our findings, and will be happy to revise the discussion to include the studies which might have skipped our attention, such as the ones mentioned by the reviewer.

Comment by reviewer: *A related question: was there life before the BMJ? It seems likely this US manuscript has been rejected by JAMA or NEJM or both, for reasons related to the points above. Yet the discussion in the paper still does not build in the reflections on causal interpretation that are needed. Despite the fact that it would be 'cool' to show that red meat is bad for health, and to strengthen the*

case that the ecological disaster of intensive beef production is not justified on human health grounds, the findings of this study are too open to alternative interpretation to provide that evidence.

Response: AS described above, we don't believe that any one observational study should make strong causal inference on its own and will require summarizing the full scope of the world's literature. Regarding the comments on beef production, here we are focused on testing the associations under study.

Comment by reviewer: *The results of the "addition models" (Supplementary Table 2) are adjusted for total energy intake while total meat intake is not held constant. This effect size from these models can be interpreted as a substitution of the meat source in question with non-meat food sources. To estimate the effect of increased intake of meat the model should adjust for the sum of energy intake from all other sources rather than the total energy intake (energy partition method).*

Response: In the addition models, the sum of the meat variables in the model equal total meat intake, so we are not sure how the inclusion of energy intake in the model should result in replacing meat with non-meat sources. However, we will be happy to address this issue, if the reviewer can provide more details about how this might happen.

Comment by reviewer: *Subgroup analysis (Figure 2) mentions the association is stronger for never smokers and never/mild alcohol drinkers. This is true for red meat but not white meat.*

Response: We want to assure the reviewer that these particular interactions were significant for both red and white meat.

Comment by reviewer: *Typo in title of Table 1: should be quintiles.*

Response: This is true, and we correct the title.

Comment by reviewer: *The idea that white meat should be substituted for red is promoted (e.g. see Abstract conclusion), perhaps accidentally, in the manuscript. There is no need to eat meat for health reasons, and this substitution idea needs no encouragement.*

Response: We agree. It was not our intention to encourage eating meat, but only to show that if (and only if) people find it difficult to reduce meat intake in general, even a change in the meat composition can be useful. We can edit to remove any sign of encouragement to use meat.