

**\*\*Report from The BMJ's manuscript committee meeting\*\***

1. As with all dietary studies, we worry that the assessment of outcome (CHD) and exposure (FFQ) seem to have a lot of uncertainty.

*We thank the committee members for giving us the chance to discuss this manuscript further. As mentioned in the manuscript, myocardial infarctions were further confirmed by physicians who were blinded to the participants' exposure status and reviewed medical records documenting symptoms and either diagnostic electrocardiographic changes or elevated cardiac specific enzyme levels. For those whose medical records were unavailable, the diagnosis was considered probable if supported by telephone interview or additional corroborating information. We did a sensitivity analysis after excluding these probable cases (10.3% of total cases), and the results were very similar.*

*Deaths were identified from searches of vital records, the National Death Index, and reports by the participant's next of kin or the postal system. This method has been tested earlier in the Nurses' Health Study cohort where the sensitivity of the National Death Index was 98% (Rich-Edwards JW, Corsano KA, Stampfer MJ. Test of the National Death Index and Equifax Nationwide Death Search. Am J Epidemiol. 1994;140(11):1016-9. PubMed PMID: 7985649). Even if a small number of certain deaths were missed, it should cause a non-differential measurement error and would likely bias the associations towards the null.*

*As for the FFQ and as discussed in the manuscript, it has been previously validated, and the correlations between FFQ and multiple dietary records were 0.59 for unprocessed red meat, 0.52 for processed red meat, 0.48 for poultry, 0.74 for fish, 0.56 for eggs, 0.62 for each of high- and low-fat dairy, 0.46 for legumes including soybeans and tofu, 0.45 for nuts, 0.27 for whole grains. In addition, we have used the cumulative average of multiple measurements which reduces random measurement errors caused by within person variation and changes in food intake over time. This measurement error, as mentioned in the limitation section, would have underestimated, not overestimated, the true associations with red meat.*

2. There is also concern about generalizability since this a group of male only health-related professionals. Might you comment more on that?

*We thank the reviewers for this important note. We did acknowledge this limitation in the manuscript as this would affect the generalizability of our findings, but not the internal validity. On the other hand, the homogeneity in this cohort would have helped in minimizing unmeasured confounding due to socioeconomic status levels. On the other hand and based on a previous study done in Nurses' Health study (NHS) cohort, there is no reason to believe that the association might be different among women, as our group has published similar associations with incidence of CHD among NHS participants (Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE, Willett WC. Major dietary protein sources and risk of coronary heart disease in women. Circulation. 2010; 122(9):876-883. doi:10.1161/CIRCULATIONAHA.109.915165) and the associations of red meat consumption with all-cause, CVD and cancer mortality were also similar among NHS and HPFS participants (Pan A, Sun Q, Bernstein AM, et al. Red meat consumption and mortality: results from 2 prospective cohort studies. Arch Intern Med. 2012;172(7):555-563. doi:10.1001/archinternmed.2011.228).*

*This was added accordingly to the limitation section.*

3. Our statistician commented that the analysis seems well done and thought out. His only minor request is that in Table 1, would you please present n and % for categorical variables.

*Table 1 has been modified accordingly.*

4. Although a reviewer asks for relative risks, we see these in the supplementary material. Perhaps they should be given more prominence in the paper itself.

*We thank the committee members for noting this. More data on HR (95% CI) were included in the manuscript.*

5. In the methods section, could you please share what the barriers were to patient and public involvement? Please also include a dissemination plan, e.g. how will you share the results of the study with patients and the public? This could include blogs, press conferences, etc.

*We thank the committee members for this suggestion. The section on Patient and public involvement has been modified as follows: "No participants were involved in setting the research question or the outcome measures, nor were they involved in the design and implementation of the study. We plan to disseminate these findings to participants in our annual newsletter and to the general public in a press release".*

#### **Reviewer: 1**

This is a first class piece of work. It will be of much interest to readers of the BMJ. However there are a few minor corrections that are needed.

*We thank the reviewer for the very insightful suggestions and positive feedback.*

1. Page 12, line 1, the heading is rather misleading. A more appropriate heading is: Comparison of findings in relation to other studies.

*We thank the reviewer for this suggestion. We have significantly edited this section based on reviewers' suggestions. The title was also changed into: "Comparison with other studies".*

2. Page 13, line 45, the main implication of the findings is that people should reduce their intake of red meat in order to help prevent CHD. It can also be mentioned that this is also good for the environment.

*We thank the reviewer for this important note. This has been added to the conclusion as follows:*

*“These findings are consistent with the effects of these foods on LDL cholesterol and support a health benefit of limiting red meat consumption and replacement with plant protein sources; this would also have important environmental benefits. <sup>36</sup>” where we also cited the following Lancet report: “Willett, W., et al. (2019). "Food in the Anthropocene: the EAT Lancet Commission on healthy diets from sustainable food systems." The Lancet 393(10170): 447-492.”*

3. Page 14, lines 18-20, the words (“whose socioeconomic status may not represent the overall population”) should be written as follows: “whose socioeconomic status is almost certainly well above the overall population”.

*This was adjusted as per the reviewer’s suggestion.*

4. Many hyphens are missing. Examples are low fat, low density, follow up. Hyphens are sometimes used but in an inconsistent way.

*This was adjusted as appropriate.*

## **Reviewer: 2**

*We thank the reviewer for their very insightful suggestions.*

### Comments:

This manuscript is about Red Meat Intake and Risk of Coronary Heart Disease Among US Men. Red meat and processed meat are very timely subjects, as well as meat versus plant product and protein sources.

1. First, for the less informed reader it is important to understand that any association between red meat, or the replacement thereof, with CHD is only about CHD. Therefore excluding e.g. quality of life or all-cause mortality. All too often this is taken as an absolute measure. While avoiding just 'the risk of CHD' is a rather strange idea, however common.

*We thank the reviewer for the comment. Indeed, the aim of this manuscript was to study red meat consumption, and its replacement with other sources of proteins, in relation to the risk of coronary heart disease. Our research group had previously conducted different studies to understand this association with all-cause, CVD and cancer mortality, and the results do support the same conclusion that red meat consumption is a risk factor for major health outcomes. While all-cause mortality is an attractive endpoint, it is biologically extremely complex because many diseases contribute to it and is also more susceptible to confounding because factors related to early detection and treatment may be difficult to control.*

*(Pan A, Sun Q, Bernstein AM, et al. Red meat consumption and mortality: results from 2 prospective cohort studies. Arch Intern Med. 2012;172(7):555-563.*

*doi:10.1001/archinternmed.2011.228; Zheng Y, Li Y, Satija A, et al. Association of changes in red meat consumption with total and cause specific mortality among US women and men: two prospective cohort studies. BMJ (Clinical research ed) 2019;365:l2110. doi: 10.1136/bmj.l2110).*

*In our models, we did account for important lifestyle factors such as physical activity, BMI, alcohol consumption and smoking which are considered important determinants for quality of life. The associations observed between red meat and CHD risk were therefore independent of these lifestyle factors. We do appreciate that additional analyses with quality of life as the outcome would be of interest, but having a fatal or nonfatal myocardial infarction would adversely affect this outcome.*

2. Second, there appears already a lot of literature on this topic, as well as mechanistic evidence, therefore the information in itself (about red meat) is not new. It is assessed in a specific group of people, however in a sound way and with multiple additional analyses. Moreover, the replacement of red meat by plant products, albeit statistically, appears to be the sensible information, as this provides the extrapolation to 'general healthy eating' beyond the endpoint of CHD.

*We totally agree with the reviewer. This paper is meant to improve our understanding of the topic while using a methodology that could reduce many of the biases present in earlier studies, and the substitution analyses are important for methodological and practical reasons.*

3. Third, it is rather strange that no word is mentioned considering interpretation of the HRs for processed red meat. What were the expectations, and why do we observe hardly any difference between processed and unprocessed red meat?

*We thank the reviewer for raising this important note. Indeed, many previous studies showed that processed meat intake was associated with worse health outcomes, and these associations were stronger in some studies as compared to unprocessed red meat. However, some of these studies had limitations which we discussed in our manuscript, and most importantly may have not adequately adjusted for total energy intake and important lifestyle confounders which may have overestimated the association of processed red meat with health outcomes. Unhealthy eating habits which are associated more with processed red meat intake would tend to overestimate the processed red meat association with CHD risk, if these behaviors were not accounted for in the model. In addition, a recent study analyzing the AARP data showed that unprocessed red meat was associated with a slightly higher risk of mortality due to heart disease, than was processed meat intake ( Etemadi A, Sinha R, Ward MH, et al. Mortality from different causes associated with meat, heme iron, nitrates, and nitrites in the NIH-AARP Diet and Health Study: population based cohort study. *BMJ (Clinical research ed)* 2017;357:j1957. doi: 10.1136/bmj.j1957). This was added to the discussion section.*

4. Fourth, while it is stressed throughout the manuscript that red meat is associated with an increased risk of CHD, it is rather the very high intake of red meat that appears to be associated with risk of CHD (only Q5 for the fully adjusted model). While we are not informed about the range of servings, the median serving of Q4 is 1.14 and Q5 is 1.72. Therefore the risk mainly lies with an intake of over 1.5 serving of red meat a day. If the authors like to overrule this information with the other analysis of HR per serving, they have to be more clear why and explain it to the reader. This may also explain the so called (introduction) inconsistency that this is not observed in populations with low red meat intake. This is not mentioned in the discussion.

*We thank the reviewer for this important note. We agree that having precise estimates of risk for each quintile would be desirable, but we expect the risk to be lower in these categories than in the highest category so a much larger sample size would be needed. Therefore, we do not have enough data to ascertain whether there is a specific cutoff point below which red meat consumption may no longer be a risk factor for CHD. While we present HR's for individual quintiles to give a sense of the quantitative association between red meat intake and risk and whether there is serious deviation from a linear dose response relation, the overall trend (e.g., HR per serving as a continuous variable) provides the strongest test of the hypothesis, and this is a standard way to present findings in this type of study. It also facilitates comparison among studies and summary statistics in future meta-analyses.*

5. Fifth, the message of less red meat and more plant protein sources is fine and fits in all current messages on healthy eating. However, many consumers that like to respond may tend to replace red meat by poultry, or even fish and eggs. Next to stressing plant protein sources as advantageous, stressing that poultry is not a good option should be considered. Maybe even that dairy is indeed helpful. The authors must know how impactful their publications are, the science is great, but you can also help in the gradual (!) transition towards healthy eating.

*We thank the reviewer for this suggestion. Since the main analysis show that the substitutions of dairy products for red meat (total, processed, and unprocessed) were associated with lower risk of CHD, we have added this to the abstract conclusion.*

*The abstract now reads as follows:*

*Results: During 1,023,872 person-years of follow-up, we documented 4,456 cases of incident CHD of which 1,860 were fatal. After multivariate adjustment for dietary and nondietary risk factors, total, unprocessed, and processed red meat intake were each associated with a modestly higher risk of CHD (HR per one serving/day increment : 1.12; 95% CI, 1.06 to 1.18 for total, HR=1.11; 95% CI, 1.02 to 1.21 for unprocessed, and HR=1.15; 95% CI, 1.06 to 1.25 for processed red meat). Compared to red meat, plant protein sources (nuts, legumes, and soy) were associated with lower risk of CHD. The HR's (95% CI's) were 0.86 (0.80 to 0.93) for 1 serving per day of plant protein sources compared with total red meat, 0.87 (0.79 to 0.95) compared with unprocessed red meat, and 0.83 (0.76 to 0.91) compared with processed red meat. Substitutions of whole grains and dairy products for total red meat and eggs for processed red meat were also associated with lower CHD risk.*

*Conclusions: Substituting high-quality plant foods such as legumes, nuts, or soy for red meat may reduce risk of CHD. Substituting whole grains and dairy products for total red meat and eggs for processed red meat may also reduce this risk.*

Minor points  
methods

6. p5 line 15: FFQ is a relatively weak dietary intake assessment method, it may not be ideal for distinction of processed meat; there is some discussion, mainly the considerable advantage of repeated dietary assessments, which is great by the way; the authors could e.g. argue that for red meat it is in fact fine?

*We thank the reviewer for raising this concern. Based on our previous dietary validation study (Hu FB, Rimm E, Smith-Warner SA, et al. Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. The American Journal of Clinical Nutrition 1999;69(2):243-49. doi: 10.1093/ajcn/69.2.243) comparing our FFQ to two 7-day dietary records, the correlations were 0.59 for unprocessed red meat and 0.52 for processed red meat. This slight difference in these correlations show that our FFQ captures almost equally processed and unprocessed red meat.*

7. p5 line 55: correlation of FFQ with multiple records is relatively low, this is not discussed; if in fact the relationship is dose dependent, the quantification of the exposure (red meat) deserves some discussion.

*We thank the reviewer for raising this point. Correlation coefficients of 0.4 to 0.7 are typically seen in dietary validation studies and may seem low to scientists accustomed to seeing higher correlations with laboratory measurements made under highly controlled conditions. However, this range of correlations is commonly seen in other epidemiologic measurements made among free living subjects over a period of months or years, including physical activity, which have well established associations with disease risk. (Willett WC, Lenart EB. Chapter 6. Reproducibility and Validity of Food-Frequency Questionnaires. In: Willett WC, ed. Nutritional Epidemiology, Third Edition. New York: Oxford University Press, 2013). Although we have reduced measurement error in the assessment of long term diet by using repeated administrations of the FFQ, we have mentioned in the Discussion that measurement error has likely led to underestimation of true associations.*

8. p10 line 52: plant based protein exchange for red meat shows stronger associations in older men; this observation is not discussed.

*We thank the reviewer for pinpointing this out. We have added the following in the discussion section:*

*“In our analysis, high quality plant-based protein foods such as nuts, legumes, and soy foods were associated with lower risk of CHD when compared with red meat. Such replacement would not only decrease the amounts of saturated fats, cholesterol, and heme iron, but also increase intake of unsaturated fat, fiber, antioxidants, polyphenols, and many constituents that could reduce risk of CHD. A reduction in CHD risk with such substitution could be therefore due to multiple changes in intakes of nutrients and phytochemicals. Since hypercholesterolemia, oxidative stress, and endothelial dysfunction increase with aging, people above 65 years of age may be at a higher risk of developing cardiovascular morbidities. Substitution of plant-based proteins for red meat could possibly improve the cardiometabolic profile of this “high-risk” group and consequently lower their CHD risk, thus explaining the more favorable substitution effect of plant-based proteins seen among older men”.*

9. p5 line 17: exclusion at baseline assumed, previous line is not about baseline, please specify.

*Correct. The phrase has been clarified as follows in the manuscript:*

*“Dietary data were not included if participants left more than 70 items blank in the FFQ or had implausible total energy intake (<800 kcal or > 4200 kcal per day) in any of the FFQs. Participants were excluded if they had a history of cancer (n=1645), myocardial infarction, angina, coronary artery bypass graft (n=3696), or stroke (n=221) at baseline.*

10. p5 line47: it may not be clear to readers why a hamburger is processed (cooked etc), and at the same time in the unprocessed category.

*In our cohorts, the Health Professionals Follow up Study and Nurses Health Study cohorts as well as in other cohorts like the Women Health Initiative (Song Y, Manson JE, Buring JE, et al. A Prospective Study of Red Meat Consumption and Type 2 Diabetes in Middle-Aged and Elderly Women. The Women’s Health Study 2004;27(9):2108-15. doi: 10.2337/diacare.27.9.2108), hamburger (lean, extra lean, regular) has been considered an unprocessed red meat. Processed red meat would include red meats preserved by smoking, curing or salting, or with the addition of chemical preservatives.*

*It is however possible that some misclassification could have occurred in this categorization. But since this misclassification is independent of the outcome, it would likely attenuate the observed association and therefore it would underestimate not overestimate the true association of processed red meat with CHD risk. Such misclassification bias was discussed in the updated limitation section.*

11. p12 line1: this paragraph is hardly providing "Strengths and weaknesses in relation to other studies", it is a direct comparison with other finds.

*We have modified this section accordingly.*

12. p14 line 14: it is not clear why the measurement error of dietary assessment would underestimate the true effect of red meat, please specify.

*Some misclassification could have occurred in the dietary assessment. For example, misclassifying unprocessed and processed red meat or inaccurate assessment of red meat in the mixed dishes. Because of the prospective study design of this study, any measurement error is likely to be independent of the outcome and would therefore attenuate the observed associations and bias it towards the null. This was added accordingly to the limitation section and reads as follows:*

*“Inevitable measurement error in dietary assessment leading to inaccurate assessment or misclassification bias, even though reduced by using the average of repeated assessments, would have tended to underestimate the true associations with red meat. Because of the prospective study design of this study, any measurement error would likely to be independent of the outcome, and therefore would attenuate the observed associations towards the null”.*

### Reviewer: 3

#### Comments:

General comments: This manuscript by Al-Shaar and colleagues studies the association between intake of total, unprocessed, and processed red meat and risk of incident coronary heart disease (CHD) in the Health Professionals Follow Up Study cohort. The strengths include a large sample size with repeated dietary assessments and comprehensive analyses. However, there has been extensive work in the literature on this topic. Hence, it is important to address nuances to the existing evidence and interpret the results in ways to inform future directions in this line of work.

*We thank the reviewer for their very insightful suggestions.*

1. Given the study population consisting of health professionals, a population that is not representative in the US population, it will be helpful to present absolute risk in addition to the current results, so that the readers will have a better understanding of the actual magnitude of the relationship described.  
*We thank the reviewer for this suggestion. Our results were presented as Hazard Ratios using cox regression models. Based on the literature and in order to be consistent with other studies, we prefer to present these results as Hazard Ratios not as absolute risk. We appreciate that absolute risks have value, but because they are so strongly dependent on the age structure of the population, they are highly non-generalizable; this is a fundamental reason why HR's or RR's are standard in epidemiologic research. Based on the committee's suggestion, we made sure that these HRs were given more prominence in the results section. The tables also included information about the number of events/person years for each quintile of red meat consumption so readers can have a sense of the absolute risks if they desire.*
2. The increased risk of CHD per serving of red meat daily is modest, ranging from 11%-15% in different types. It is important to explain why this is the case; and why the differences between unprocessed and processed red meat are small (4%), as we would expect a greater risk difference in processed meat which contains nitrates and a higher level of sodium. Also, the results suggest a higher risk in unprocessed than processed red meat for fatal CHD and for total CHD after controlling for the dietary quality index. These findings deserve further discussion to interpret the results. The earlier systematic review by Micha et al. (2010) published in Circulation reports different findings on the same topic.  
*We thank the reviewer for raising this point. It is true that the increased number of meat servings per day was associated with 11% to 15% increased risk of CHD. While this could look a relatively small increase in the risk but knowing the high prevalence of CHD in the US population, the impact of this consumption would be large at a population level. Also, it is true that the higher risks associated with processed and unprocessed red meat consumption were not too different from each other as compared to other studies. This could be because we accounted for other dietary factors including other sources of proteins, fruits, and vegetables, glycemic index, as well as other important lifestyle factors such as physical activity, smoking and alcohol intake, which most of the times have not been accounted for in other studies. Accounting for these lifestyle factors could have indirectly adjusted for some unmeasured confounders such as unfavorable eating habits which maybe more prevalent among high consumers of processed red meats. High sodium and preservatives content of processed red meats may partly but not entirely explain the increased risk of CHD. Constituents of red meat present in both processed and unprocessed red meats such as saturated fats, dietary heme iron, L-carnitine, sialic acid N-glycolylneuraminic acid (Neu5Gc) and others maybe also driving the increased risk of CHD. A more detailed mediation analysis using other detailed datasets would be needed*



*to explore this question further. Also, the benefits of replacing red meat with plant sources of protein likely come in part from beneficial components of these foods, as we have included in the Discussion.*

*Upon adjusting for the alternative healthy eating index which accounts for the overall diet quality, the association was attenuated but remained significant. This clearly shows that associations between red meat and other outcomes might be overestimated in other studies, particularly if these studies did not comprehensively adjust for the overall quality of diet and lifestyle behaviors.*

*Also, it true that our findings are different from the earlier systematic review and meta-analysis of Micha et al study (2010). Such discrepancy may be attributed to the fact that only 4 observational studies were included in the analysis of 2010 (769 cases only): Burke et al included dietary data that focused on animal sources only. They had a total of 118 CHD events out of 514 participants. Ascherio et al, used data from the Health Professionals Follow-up Study cohort, which only had 4 years of follow-up (until 1990), and the number of cases was consequently small (386 out of 44,933). Martínez-González et al study was a case control study (171 cases, 171 controls) and not a prospective cohort, where patients admitted for MI were included and matched controls were selected from other hospital wards. Recall bias would be important to consider in such a study design. Whiteman et al had however a larger sample although still small (94 cases out of 10,522), but similar to many other studies, their models were not isocaloric (i.e. adjusted for total energy intake), and the comparison food was not specified. In our study, we studied 4,456 cases and used isocaloric models while taking into account all other sources of proteins and calories. The comparison foods were explicitly specified in our models, unlike other studies which implicitly compare the food(s) under study with the rest of all other calorie contributing foods of the diet which are typically largely from refined starch, sugar, potatoes, and saturated fats. This discrepancy was further evaluated in the section on “Strengths and Limitations in relation to other studies”.*

3. The analyses include many exposures of interest and multiple outcomes. I think the 2-sided p-values may require a smaller value less than 0.05 if Bonferroni correction for multiple comparisons is not possible. Can you please add p-values for the results? Currently, only p for trend is presented.

*We thank the reviewer for this note. Since our hypothesis is testing the overall association, the issue of multiple comparisons is therefore reduced, and therefore we think there is no need to report the p-values per each quintile. We have however included below the p-values of Table 2, for the reviewer to check, and we could include these p-values if the editors so wish.*

*As for the substitution analysis, p-values of Figure 1 were added to Supplementary Table 4, and those for dairy products and fish were added to Tables 3 and 4.*

**Table 2:** Hazard Ratios (95% CI) for total CHD associated with quintiles of total, unprocessed, and processed red meat intake (N=43,271).

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	HR per 1 serving/d	P-trend*
<b>Total red meat</b>							
Median servings/d	0.21	0.52	0.78	1.14	1.72		
Cases/PY	811/203879	833/206108	859/203718	865/206087	1088/204079		
Age-adjusted Model	1	1.08 (0.98, 1.19); p=0.12	1.15 (1.04, 1.26); p=0.004	1.15 (1.04, 1.27); p=0.004	1.47 (1.34, 1.61); p<0.001	1.20 (1.16, 1.26); p<0.001	<0.001
Multivariable-adjusted Model 1	1	1.06 (0.96, 1.17); p=0.23	1.11 (1.00, 1.23); p=0.04	1.09 (0.97, 1.21); p=0.09	1.34 (1.21, 1.49); p<0.001	1.15 (1.09, 1.21); p<0.001	<0.001
Multivariable-adjusted Model 2	1	1.06 (0.96, 1.18); p=0.23	1.11 (0.99, 1.23); p=0.05	1.08 (0.97, 1.21); p=0.18	1.28 (1.14, 1.45); p<0.001	1.12 (1.06, 1.18); p=0.001	<0.001
<b>Unprocessed red meat</b>							
Median servings/d	0.14	0.35	0.5	0.71	1.09		
Cases/PY	847/205918	876/199361	840/207111	877/201942	1016/209540		
Age-adjusted Model	1	1.13 (1.02, 1.24); p=0.01	1.08 (0.98, 1.19); p=0.13	1.17 (1.06, 1.28);p=0.001	1.36 (1.24, 1.49); p<0.001	1.27 (1.18, 1.35); p<0.001	<0.001
Multivariable-adjusted Model 1	1	1.12 (1.01, 1.23); p=0.03	1.05 (0.95, 1.16); p=0.34	1.12 (1.01, 1.23); p=0.03	1.24 (1.12, 1.37); p<0.001	1.17 (1.08, 1.26); p<0.001	<0.001
Multivariable-adjusted Model 2	1	1.11 (1.01, 1.22); p=0.04	1.04 (0.94, 1.16); p=0.42	1.09 (0.98, 1.22); p=0.10	1.18 (1.05, 1.32); p=0.005	1.11 (1.02, 1.21); p=0.02	0.01
<b>Processed red meat</b>							
Median servings/d	0.02	0.14	0.21	0.38	0.71		
Cases/PY	889/224469	734/181661	883/211353	843/201440	1107/204950		
Age-adjusted Model	1	1.05 (0.95, 1.15); p=0.36	1.14 (1.04, 1.25); p=0.006	1.12 (1.02, 1.24); p=0.02	1.39 (1.27, 1.52); p<0.001	1.32 (1.24, 1.41); p<0.001	<0.001
Multivariable-adjusted Model 1	1	1.02 (0.93, 1.13); p=0.66	1.09 (0.99, 1.20); p=0.08	1.06 (0.96, 1.16); p=0.28	1.24 (1.12, 1.36); p<0.001	1.20 (1.12, 1.30); p<0.001	<0.001
Multivariable-adjusted Model 2	1	1.02 (0.92, 1.13); p=0.71	1.09 (0.98, 1.20); p=0.10	1.05 (0.95, 1.17); p=0.33	1.19 (1.07, 1.33);p=0.002	1.15 (1.06, 1.25);p=0.001	0.001

4. Also, there are many confounders adjusted in the model. After controlling for the dietary variables, the effect size attenuates. This is especially the case after adjustment for the healthy eating index shown in the supplemental results. Is controlling for AHEI for dietary quality a more conservative approach to use in this study?

*We thank the reviewer for this note. AHEI would not be considered a more conservative approach, but an approach to account for the overall diet quality and reduce possible unmeasured confounding. There was only a slight decrease in the HRs upon including the modified AHEI into the model. This gives more confidence in the robustness of our study findings.*

5. In the substitute analyses, besides the different sources of plant-based foods, dairy products were also shown to have a beneficial association. The conclusion of the Abstract emphasized the protective association in plant-based protein. I think there are two problems here: 1. Plant-based protein is not the main exposure of interest in this study. Would that be more appropriate to summarize the findings for red meat consumption? 2. Plant-based protein did not show any association with CHD in participants aged younger than 65 years. Hence, the authors may want to carefully draw the conclusion in the Abstract as well as in the manuscript and point out this discrepancy in Results and Discussion.

*We thank the reviewer for this important note. We have adjusted the abstract and conclusion to better reflect the findings of our study. We did a priori hypothesize that replacement with plant sources of protein would be associated with lower risk because of benefits of this replacement on LDL cholesterol. Results of the stratified analysis by age and total fiber intake were included in the discussion and conclusion, but not the abstract as these were not prior hypotheses.*

Abstract:

6. Please check Results. There are a couple of typos there.

*We could not find any typos, but maybe the BMJ copy editors can sniff them out.*

7. Introduction:

8. Lines 17-31: I find it helpful if the authors can cite references to support the inconsistencies found in the literature. The argument made here is not convincing. I also find it difficult to understand the last two sentences in Lines 27-31.

*New references were included about possible causes for the inconsistencies observed among the different studies of red meat.*

*Lines 27-31 describe the issue when researchers do not explicitly specify the food group which the red meat is being compared to. If the comparison group is not specified in the model, the researchers would be comparing red meat to the remaining diet which is often rich in refined carbohydrates and unhealthy fats. Comparing red meat to an unhealthy comparison group would often show that red meat is not a risk factor for CHD, and inconsistencies might therefore arise across such studies.*

9. Line 35: please specific “these issues.”

*This was adjusted into: “To address these issues in study design and analysis”.*

10. Page 5, Line 46: poultry is missing. Was seafood part of fish in the FFQ?

*Thank you for pointing this out. We have adjusted this accordingly. As for fish, the following was included in the definition: canned tuna fish, breaded fish cakes, pieces, or fish sticks, other fish like cod, haddock, halibut, in addition to dark meat fish such as salmon, swordfish etc. We did not include shellfish.*

11. Page 8, lines 4-5: is it possible to conduct stratified analysis by BMI ( $\geq 25$  and  $< 25$ ) and by fiber intake ( $\geq 28$ g/d and  $< 28$ g/d)

*We thank the reviewer for the suggestion. We have conducted the new analysis but did not find a significant interaction with BMI. However, significant interactions were noted between fiber intake and plant-based proteins, legumes, and nuts. The substitution effects of these food groups for total red meat were stronger among those who had higher fiber intake. The associations of nuts and plant-based proteins were attenuated but remained significant among those whose fiber intake  $< 28$  g/day. The new analyses are now included as a Supplementary Figure 2.*

12. Page 8, lines 12-22: as repeated dietary assessments are available, it will be helpful to comment whether there is any difference in red meat consumption at baseline, the most recent diet and the cumulative average in the Results.

*There was not a big difference in total red meat consumption when using the baseline data (0.99 servings/day  $\pm$  0.73), recent diet (0.87 servings/day  $\pm$  0.72) or the cumulative average (0.91 servings/day  $\pm$  0.64). Yet, it is important to note that the standard deviation was smaller when multiple measurements were used for calculating the cumulative average, thus further reducing random measurement errors. These new information were added to the results and discussion sections.*

13. Page 9, lines 31-33: please specify the units or categories of the dietary variables adjusted for in the model.

*We have included this information in the “statistical analysis” section.*

14. Page 9, line 42: “a one serving per day”?

*The phrase was edited as follows: “For an increment of one serving per day”.*

15. Page 10, line 37: I find dark meat fish is an unusual term. On this line, tuna is part of it. But in Supplementary Table 6, tuna is in its own category. Why is this the case? Is it possible to use a more general term such as fatty fish and non-fatty fish? Also, does fish include seafood in this study?

*In our analysis, total fish did not include shellfish in our definition. Since the substitution effect was unexpectedly not significant with fish, we wanted to examine the different types of fish which could possibly be contributing to the direction of the association. So, in a sensitivity analysis that further examines the different types of fish, we saw that substitution of dark meat fish for red meat was associated with lower CHD risk after the calendar year 2000. This could be due to the variation in the method of food preparation over time, as fish were mostly consumed as deep fried in the earlier years. On the other hand, there was a typo in Supplementary Table 1 which was corrected to reflect the different types of fish included under dark meat fish.*

16. Page 10, lines 48-54: please add the results of plant-based protein sources for men younger than 65 years.

*Done.*

17. Page 11, lines 12-14: please comment on why a weaker association was found in the more recent diet in Discussion.

*We thank the reviewer for this point. We added the following to the discussion:*

*“Also, associations were stronger using cumulative average intakes than with single dietary assessments, likely reflecting the less precise measurement of long-term diet when using a single questionnaire compared with using the cumulative average of repeated assessments. This was consistent with the larger standard deviation of total red meat intake observed when single measurements were used (baseline and most recent diet) as compared to using the cumulative average of multiple measurements. The weaker associations observed when using the most recent dietary data may also be influenced by reverse causation bias as participants could have changed their diet after developing symptoms or a diagnosis related to cardiovascular disease”.*

18. Page 11, lines 35-37: I think this should be clear that the protective association was only found in men older than 65 years. Also, I find the term “high-quality plant-based protein” confusing. How was the quality of plant-based protein determined? Were other sources such as quinoa and seeds included in the estimated intake of plant-based protein? If only a few groups (nuts, legumes and soy) were assessed, please say so.

*The first paragraph of the discussion was modified accordingly. As for the quality of plant-based proteins, this included the major sources of proteins which come from nuts, legumes and soy. We did not have specific questions on quinoa as intake has been low in this cohort or other seeds (nuts, legumes, and soy are all seeds). We have included in the “dietary assessment” section a phrase that explicitly explained what was included in this “plant-based food group”.*

19. Page 12, line 1: the subtitle “Strengths and weaknesses in relation to other studies” does not seem appropriate. Why would these be strengths and weakness when the results are compared with those in the existing literature?

*This section was modified accordingly.*

20. Page 12, line 39: I find these mechanisms are not helpful or not directly connected to the findings in this paper. For example, it points to fats and cholesterol are responsible for the risk of CHD, but red meat is a whole food. Or if heme iron is culprit, I think iron intake should be tested to see its association with CHD risk. For TMAO, it can come from red meat, eggs, dairy products, and salt-water fish? So this speculation did not make sense here, as the relationship for eggs, dairy products and fish tend to have a protective association in the analysis.

*We thank the reviewer for this important observation. The aim of this section was to explain potential mechanisms linking red meat to CHD. It is true that red meat is a whole food but reducing its intake in an isocaloric medium would mean increasing the intake of other protein sources. This would lead to changing the overall intake of dietary cholesterol, saturated fats, antioxidants, polyphenols, and other dietary components. Therefore, any reduction in CHD risk could be therefore due to multiple changes in the intake of the different nutrients and phytochemicals.*

*Heme iron may not be the only culprit because its concentration is known to be lower in processed than unprocessed red meat, and therefore its mediation effect would be interesting to explore in other future studies. As for TMAO precursors, since we are comparing serving to serving, amounts of these precursors would differ from one food group to another, and their effect on atherosclerosis might be modified by other dietary constituents present in the comparison food. We agree that the entire findings cannot be accounted for by TMAO precursors, or any single mechanism, but the combination of multiple factors in the whole foods, and in the substituted foods, is most likely an explanation. A more detailed discussion of potential mechanisms would greatly lengthen this section of the manuscript.*

21. Page 12, lines 37-39: this statement needs a reference, please.

*The whole section on “comparison with other studies” was edited.*

22. Page 14, lines 16-20: “may not”?

*This was modified according to the other reviewer’s comments.*

23. Page 14, lines 31-33: The results suggest that other animal sources such as eggs and dairy products also showed a significantly lower risk for CHD, and plant-based protein was protective in men older 65 years. Please be accurate in the concluded statement.

*This was adjusted accordingly.*

24. Page 16, lines 23-30: for the added information: first, I don't think this topic is new; second, the stratified analysis shows only plant-based protein is protective in men older than 65 years. Perhaps add something more nuanced from the findings.

*The section was edited as such:*

*-Compared with total, unprocessed, or processed red meat, other dietary components such as soy, nuts, and legumes were associated with lower risk of CHD.*

*-Substitutions of whole grains and dairy products for total red meat and eggs for processed red meat were also associated with lower CHD risk.*

*We did not add the findings of the stratified analysis by age or fiber intake as this was not an a priori hypothesis.*

Reviewer: 4

Comments:

Meat Intake and Risk of Coronary Heart Disease Among US Men

Thank you for this study on an interesting and relevant subject. The paper is well read, the analyses are solid and well performed, and the conclusions are clear. Specifically, using the substitution models reveal very interesting results. Below some suggestions for improvement. I would especially try to make a better focus in statistical analysis and results towards your research question so your analysis are really focused on answering the question, which will make it easier to read.

*We thank the reviewer for their positive feedback.*

Page 1:

-Title: In your abstract and introduction it becomes clear that the main new information your study adds is that plant based protein sources are a good substitution for red meat. You could consider to add this in the title. Especially since meat intake is already correlated to CHD in multiple other studies as you mention in your introduction and discussion.

*We thank the reviewer for this suggestion. Because dairy products and eggs showed also to be associated with lower CHD risk as compared to red meat, we prefer to keep the title as is. We have however edited the title to include the research design of this study.*

Page 3:

-Besides plant based foods also dairy and whole grains reduce risk for CHD. Why is this not in the conclusion?

*Our primary aim was to study plant-based foods substitution for red meat in relation to CHD risk, and therefore we did not include the other food groups in our conclusion. However, based on reviewers' suggestions, we have modified the conclusions in the abstract and main text have been edited to include these food groups as well.*

Page 4:

-3-10 I would suggest to change beginning to: "Substantial evidence from randomized trials and observational studies suggests that high consumption of red meat, especially processed red meat, is associated with increased risk of mortality<sup>2-4</sup> and major chronic diseases<sup>5-10</sup>, including the coronary heart disease (CHD)<sup>11-13</sup> -14-15: 'not seen in a population'.

*We thank the reviewer for this suggestion. The text was modified accordingly.*

Page 5

-The study is performed among the Health Professional study, why is the Nurses Health study not included?

*Our research group has previously studied the association between major sources of proteins (including the red meat) and risk of coronary heart disease among participants of Nurses Health Study (Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE, Willett WC. Major dietary protein sources and*



*risk of coronary heart disease in women. Circulation. 2010; 122(9):876-883. doi:10.1161/CIRCULATIONAHA.109.915165). The relationship was very similar to our findings. Comparing the 5<sup>th</sup> quintile to first quintile of red meat consumption, HR=1.29 (1.12, 1.49), and HR for 1 serving/day =1.16 (1.09, 1.23). This is to note that the multivariate adjustment done in Bernstein et al study was very similar to Model 1 in our current study. Although substitution analyses were conducted slightly different in that paper in terms of food grouping and analysis, yet the overall conclusion was similar: Substituting red meat with other sources of proteins in the US diet was associated with lower CHD risk.*

- For those unfamiliar with the Health Professional study a few details on the population would be helpful (e.g. health status, what why health professionals, how were they recruited, region were they live). A reference to a design paper of the cohort would be helpful as well.

*We thank the reviewer for this suggestion. The methodology was edited accordingly and a reference was included for further information.*

-15-17 '70 items blank on FFQ or intake <800 or >4200'? Could you support this approach by a reference?

*The following reference was added accordingly (Hu FB, Rimm E, Smith-Warner SA, et al. Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. The American Journal of Clinical Nutrition 1999;69(2):243-49. doi: 10.1093/ajcn/69.2.243), and this method has been consistently used by our research group.*

- Why are patients with cancer excluded? And why not patients with for example Diabetes Mellites or COPD etc?

*In order to reduce biases from existing illnesses, we have excluded patients with history of cancer, as these participants are very likely to change their diet because of their disease, hence bias the association between red meat consumption and CHD risk. We did not exclude participants with other morbidities such as diabetes, hypertension, or hypercholesterolemia, as these could act as intermediate factors, on the causal pathway linking red meat consumption with CHD risk. We did however account for these comorbidities in a sensitivity analysis, as described in the manuscript.*

Page 6

-12: Why are data censored at January 31, 2016? Is there no more up to date information known on incidence CHD?

*Unfortunately, we do not have data on CHD beyond 2016 as we usually adjudicate these events through accessing participants' medical records. That's why follow-up was truncated at 2016.*

- How was physical activity assessed? Using a validated questionnaire?

*Our physical activity questionnaire has been validated previously (Chasan-Taber S, Rimm EB, Stampfer MJ, et al. Reproducibility and Validity of a Self-Administered Physical Activity Questionnaire for Male Health Professionals. Epidemiology 1996;7(1):81-86.) and correlations between physical activities reported in diaries and that in the questionnaire were 0.58 for vigorous activity and 0.41 for inactivity. We added the following in the "Assessment of covariates" section: Data on Physical activity (< 3, 3-8.9, 9-17.9, 18-26.9, and ≥ 27 in metabolic equivalents per week) were also collected using the validated physical activity questionnaire (Chasan-Taber S, Rimm EB, Stampfer MJ, et al. Reproducibility and Validity of a Self-Administered Physical Activity Questionnaire for Male Health Professionals. Epidemiology 1996;7(1):81-86.)*

*Our research group is currently validating this questionnaire using more recent data collected in Men's Lifestyle validation Study and Women's Lifestyle Validation Study comparing self-reported activities by the physical activity questionnaire to four 24-hour recalls, accelerometer, and DLW measurements.*

Page 7

- 23: please report the covariates.

*The text was modified accordingly.*

- The AHEI is not mentioned in the paragraph on covariates.

*The text was modified accordingly.*

- To improve the readability of the statistical analyses I would suggest to first clearly mention all the analyses directly required to answer the research questions. After that the sensitivity analyses can be described.

*The text was modified accordingly.*

- Did the authors consider BMI as an effect modifier or causal interference by BMI? Data are stratified for age categories, but not for BMI. It would be of value to spend more time and analyses to better understand the possible interference of BMI with the results - more than only adjusting for BMI groups in the models.

*This new analysis was also suggested by another reviewer, and we conducted the analysis and BMI was not an effect modifier. This was included in the manuscript.*

page 8

-3-5: what is the reason you stratified by calendar time and why the year 2000?

*Year 2000 was chosen as it represented midpoint of the follow-up time of the cohort, and trans-fat consumption had started to decline since then.*

27: I assume the proportional hazards assumption was made? Please make this statement.

*This was mentioned in the "Statistical Analysis" section where we tested "the proportional hazards assumption by including an interaction term between red meat intake and months to events".*

Page 9

-Please give some demographics about participants in first sentence of the results or minimally average age and age range.

*We thank the reviewer for this suggestion. The section was edited accordingly.*

Page 10

- 29: The novelty of this paper mainly lies in the substitution models. In the results, the HR for plant protein sources is mentioned. Yet for dairy and fish we are redirected to the supplementary results. I would suggest to provide these results a more prominent place in the result section.

*We thank the reviewer for this suggestion. We moved these supplementary tables to the main text (now Tables 3 and 4).*

Page 12

In the part on strengths and weaknesses in relation to other studies I do not really see how the current study is compared to other studies. It is more a summary of previous findings. But how would you explain differences and/or similarities? And are there weaknesses or strengths in the current design compared to other studies/results? E.g. do you expect other results among females? Other methods to measure diet etc?

*We thank the reviewer for this important note. This section has been significantly modified.*

Page 12

The describes mechanisms are clear and useful. However, I miss some implications of these extremely important results. For example:

-Your results provide opportunity to optimize dietary advice.

-The current situation in the world asks for serious changes in the use of resources. The observed results do not only offer plenty of opportunity to improve health, but also to do so in a sustainable manner.

-What future research steps are required before this finding can be made more ‘official’. Or is it already possible to advice policy makers, health care workers etc?

-Taking a broader view; red meat is clearly associated with higher CHD risks. What about other health outcomes? Would you expect that your results apply to other outcomes? Cancer, sarcopenia etc?

These results are very important! And I would take the opportunity to highlight the possible implications of these results in more detail (not only the conclusion).

*We thank the reviewer for their very positive input. The current study adds more evidence that red meat consumption is a risk factor for CHD. Many researchers in our group have investigated its relationship with Type II Diabetes, cancer, in addition to mortality (all, CVD, cancer mortality). This also supports the report of EAT-Lancet Commission on Food, Planet, Health on healthy and sustainable diets.*

*However, we prefer to stick to the current conclusion since we also observed lower CHD risk when substituting red meat with other animal proteins such as dairy products and eggs.*

*We have however edited the conclusion as such:*

*“We found that greater intakes of total, unprocessed, and processed red meat were each associated with a higher risk of CHD. Compared with total, unprocessed, or processed red meat, other dietary components such as soy, nuts, and legumes were associated with lower risk of CHD. These associations were stronger among older men. These findings are consistent with the effects of these foods on LDL cholesterol and support a health benefit of limiting red meat consumption and replacement with plant protein sources; this would also have important environmental benefits<sup>44</sup>. We also found that substituting whole grains or dairy products for total red meat and eggs for processed red meat were also associated with lower CHD risk. Further research on the substitution of dairy products and egg intake for red meat are needed in other cohorts to confirm the generalizability of these findings”.*

Page 13

The part on the weaknesses apply generally to all observational studies and could be made more specific to the current study. Also, how could these weaknesses have effected the results? For example, do you expect different results in other ethnicities? What kind of residual confounding can be expected (any specific covariates that are yet known to influence the association).

*In this section, the aim was only to address the possible biases that could have emerged in our dataset including its observational design. Residual and unmeasured confounding from other lifestyle behaviors are expected in these observational studies. As for expecting different results in other ethnicities, this would depend on the type and amount of meat consumed in each group, and how much red meat whether processed or unprocessed is contributing to the total diet.*

Page 14

- In the conclusion only the reduced CHD of substitute plant based foods are mentioned not dairy and whole grains, why is this?

*The primary aim of our study was to test the substitution effect of plant-based proteins for red meat. That's why the conclusion was written this way. Based on the reviewers' comments, we have modified it accordingly to also include dairy, whole grains and eggs.*

Page 16

- Based on the studies you mention in your discussion I would state that point 3 was already known and that your study confirms this. Your study especially adds new information on plant based protein sources that reduce CHD as substitution of red meat.

*The section was revised accordingly.*

Page 17

-Why is chosen to not report quantile 2 and 4?

*We chose not to report quantiles 2 and 4 in order to make the table look less busy as it currently has 10 different columns. If the journal would allow this width, we will be happy to adjust it accordingly.*

- what is the reason that for instance fruit and vegetable intake is energy adjusted and red meat intake is not?

*In all our analyses, we adjusted for total energy intake, and used the unit as serving in order to be able to have a serving to serving comparison.*

Page 19

-why is substitution of whole grains in supplementary material and not in figure 1?

*We initially tried including the HR for whole grains to the figure, but it has overwhelmed it. Because of the larger magnitude in RR reduction, it made RR reductions of 0.8-0.9 seen in other food types less meaningful, so we decided to include it in the supplementary material only. Also, if someone would want to significantly reduce their total red meat intake, they would probably think of plant-based proteins and other animal proteins. Whole grain is mostly thought of as a primary fiber source.*