Dear editor and reviewers

Thank you very much for the numerous comments and suggestions regarding our paper on whole grain consumption and cardiovascular disease, cancer and all-cause mortality. We have made changes to the manuscript taking the comments from the editors and reviewers as much as possible into account and we think the quality of the paper has improved substantially because of this. We have provided a point-by-point response to each comment below. We hope this will be sufficient for the paper to be accepted for publication in the BMJ, but are willing to make any further changes should it be necessary.

Best regards

Dagfinn Aune, MS, PhD student
Lars T. Fadnes, MD, PhD
Darren C. Greenwood, PhD
Teresa Norat, PhD

-Our statistician made the following comments:

p13/7: Why was 7 servings per day optimal? Isn’t this rather a lot???
RESPONSE: We used 7 servings per day as optimal as it was the level of intake that gave the highest reduction in risk. We agree that this is a quite high intake and for this reason we also presented attributable fractions based on a more modest reference intake of 4 servings per day as well. In the revised manuscript we therefore have focused the PAR on an intake of 4 servings per day (120 g/d).

p14/37 – 18/41: a very long and largely unreadable run through the various analyses shown in figs 2-7 plus supplementary figures. This is tough stuff to wade through – much of it simply repeats what’s shown in the figures. Not a good use of space – unreadable! Would be better in my view to have one or more tables summarising the results of all the analyses. Then I found that this is in Table 1!!

RESPONSE: Table 1 only shows the results for the subtypes of whole grains. We have added a new Table 1 with all the results for total whole grain (results for subtypes of grains are now presented in Table 2). We shortened down the section on whole grains and other causes of death so it reads: “Inverse associations were also observed for the association between whole grains and mortality from respiratory disease (Figure 5, Supplementary Figure 6, Table 1),(4;9;20;22) diabetes (Figure 5, Supplementary Figure 7, Table 1),(4;9;20;22) infectious disease (Figure 6, Supplementary Figure 8, Table 1),(4;20;22) and non-cardiovascular, non-cancer causes of death (Figure 7, Supplementary Figure 10),(4;9;20;22;27), but not for nervous system disease mortality (Figure 6, Supplementary Figure 9, Table 1),(4;20)”

“Study quality was assessed using the Newcastle-Ottawa scale (NOS) which awards 0-9 stars based on the selection, comparability, and outcome assessment.[36] We considered studies with 0-3, 4-6, and 7-9 stars to represent low, medium and high quality studies.” Summary ratings show median of 8/9 which is surprisingly high.
All studies were included in the meta-analysis regardless of methodological quality – but few of low quality it seems

RESPONSE: We did not set a certain study quality score as an inclusion criteria. Most of the included studies are relatively recent studies so the conduct and reporting of the studies may therefore have been improved and resulted in the high study quality scores. We added tables with the study quality scores for the studies of whole grains and CHD, stroke, CVD, total cancer and all-cause mortality and modified the sentence in the section on study quality in the results so it reads: Mean (median) study quality scores for the studies on whole grains were 8.3 (8.0) for coronary heart disease, 8.2 (8.0) for stroke, 8.0 (8.0) for cardiovascular disease, 7.8 (8.0) for total cancer, and 8.2 (8.0) for mortality out of a maximum of 9 points (Supplementary Table 17-21).

p18/50: There are few studies in each analysis thus low power to detect small study effects. Suppl figs

RESPONSE: We agree and modified the sentence in the discussion (last section of limitations): “There was no evidence of publication bias for the remaining outcomes, although the number of studies was moderate and power to detect such bias may have been low.”

85-89 do show some suggestion of larger effects being in small studies only.

RESPONSE: We agree, but this was more pronounced for CHD and stroke (for which Egger's test suggested publication bias) rather than total CVD, total cancer, and all-cause mortality. For the CVD and mortality outcomes it appeared to be only 1 study which caused the slight asymmetry in the funnel plots, thus chance might also be a possible explanation.

19/50: popnattrib risk calcs give very precise estimates with no CIs. I’m very sceptical about all of these calcs. No comment here about very strong (and unreasonable) assumptions – see p21 where they say “Under the assumption that the observed associations are causal”. Also unclear what is being compared to what here.

RESPONSE: We agree and have rounded the calculations to the nearest 1000. We agree with the editor that the assumption about causality is strong, but we don’t think it is completely unreasonable based on the numerous potential mechanisms that were discussed which could explain the lower risk of cardiovascular disease, and all-cause mortality, for example through reductions in blood pressure or hypertension, lower cholesterol and triglyceride levels, as well as improved glycemic control and reduced insulin resistance. We think the PAR is a useful tool to put the size of the associations into context and others have done this as well in the BMJ recently (BMJ. 2015 Jul 21;351:h3576.) and we thought it added an extra dimension to the presentation. However, we would be willing to drop it if the editors thought this an assumption too far.

They did adjust for lots of things and say it made no difference: “the associations observed persisted” but results not shown. (22/41). But these adjustments would be at the study level I assume as don’t have IPD. So are very dependent on what was adjusted for in primary studies. The NOS requires reviewers to indicate what they seek adjustment for but this isn’t
specified here. NOS doesn’t address several issues e.g. missing data.
RESPONSE: Subgroup analyses by adjustment for confounding factors were shown in Supplementary Table 15 and 16 and in general showed little evidence of heterogeneity between subgroups. Yes, it is correct that the adjustments were at the study level as we didn't have IPD. As shown in Supplementary Table 15 and 16 subgroup analyses were conducted by whether studies adjusted for age, education, family history of CHD, BMI, smoking, alcohol, physical activity, hypertension, hypercholesterolemia/serum cholesterol, coffee/caffeine intake, sugar-sweetened beverages, red or processed meat, fish, fruits and vegetables, dairy and energy intake. In the NOS assessment one point was given for adjustment for age and another point was given for adjustment for any other confounder. We agree with the reviewer that NOS doesn’t address missing data and that this is a problem for meta-analyses of observational studies.

- One editor felt that you do not make a good case for novelty in the introduction or the discussion. She was also concerned that you barely seem to mention confounding, and she could not find a clear statement about covariates (and adjustment for them) in these studies. She also did not see sensitivity analyses according to study quality, and not much discussion of study quality in general. She considered that even though this may be a useful update, she was not convinced that this was a priority for The BMJ.
RESPONSE: We have made considerable changes to the introduction and added the following section to provide a better justification and more novelty for the meta-analysis: "In spite of a growing body of epidemiological evidence for the health benefits of whole grain consumption, dietary recommendations have often been unclear with regard to the amount of whole grains that should be eaten to reduce chronic disease risk. For example in the World Cancer Research Fund 2007 report it was recommended individuals should "eat relatively unprocessed cereals (grains) and/or pulses with every meal",(25) while in the United Kingdom there is no specific recommendation other than "choosing whole grain, brown or high fibre varieties wherever you can", but no specific quantities of whole grains were recommended.(26) In the USA and Canada the recommendation is that "all adults eat at least half their grains as whole grains" so at least 3 servings of whole grains should be consumed per day,(27) while in the Scandinavian countries at least 75 grams per day of whole grain intake (dry weight) which equals approximately 250 grams per day (~8 servings/day) of whole grain products (fresh weight) is recommended (28). There may be several reasons for the inconsistent dietary guidelines for whole grain intake including difficulties in measuring whole grain intake, differences in the consumption patterns of whole grains between populations, or lack of data on whole grain intake in some populations, but they may also be because most previous meta-analyses only considered selected disease endpoints and did not conduct dose-response analyses.(5;24) Some of the current authors found a reduced risk of type 2 diabetes incidence with up to 2-3 servings per day (60-90 g/d) of whole grain intake, but no further reductions in risk with higher intakes,(4) while in a second meta-analysis of whole grain intake and colorectal cancer a linear inverse association was observed up to an intake of 180 g/d.(21) Whether the association is linear or reaches a plateau for other chronic disease outcomes and all-cause mortality, or whether only specific types of whole grains are associated with chronic disease and all-cause mortality would be important to clarify to provide more detailed and consistent dietary recommendations with regard to the amount of whole grains that should be consumed to reduce the risk of chronic disease and premature mortality. Answering this question would also clarify whether there are additional benefits with
very high intakes such as those recommended in the Scandinavian guidelines,(28) and whether such high recommendations are justified.”

Subgroup analyses according to study quality were provided in the Webappendix in Supplementary Table 15 and 16. Study quality was also mentioned in the results section: “Mean (median) study quality scores for the studies on whole grains were 8.3 (8.0) for coronary heart disease, 8.2 (8.0) for stroke, 8.0 (8.0) for cardiovascular disease, 7.8 (8.0) for total cancer, and 8.2 (8.0) for mortality out of a maximum of 9 points (Supplementary Table 17-21).” Because all the studies of total whole grains were in the highest category of study quality (Supplementary Table 15, 16) we did not discuss the issue in depth, but we added a sentence of strengths of the analysis including study quality: “Strengths of the current meta-analysis includes the comprehensive analyses of whole grain and subtypes of grain intake in relation to a range of chronic disease and mortality outcomes including high vs. low analyses, linear and nonlinear dose-response analyses, the detailed subgroup, sensitivity, and influence analyses, the large number of cases or deaths and participants included, the high study quality of the studies included as well as estimation of the population attributable risk due to low consumption of whole grains.”

Some parts of the introduction already mentioned some of the novel aspects of the study including clarifying the dose-response relationship between whole grain consumption and CHD, stroke, CVD, total cancer and all-cause mortality and less common causes of death. No previous meta-analysis has been conducted on whole grains and total cancer, all-cause mortality and less common causes of death and this was mentioned in the introduction already. We modified the introduction and have added: "There may be several reasons for the inconsistent dietary guidelines for whole grain intake including difficulties in measuring whole grain intake, differences in the consumption patterns of whole grains between populations, or lack of data on whole grain intake in some populations, but they may also be because most previous meta-analyses only considered selected disease endpoints and did not conduct dose-response analyses.(6;31) Some of the current authors found a reduced risk of type 2 diabetes incidence with up to 2-3 servings per day (60-90 g/d) of whole grain intake, but no further reductions in risk with higher intakes,(5) while in a second meta-analysis of whole grain intake and colorectal cancer a linear inverse association was observed up to an intake of 180 g/d.(28) Whether the association is linear or reaches a plateau for other chronic disease outcomes and all-cause mortality, or whether only specific types of whole grains are associated with chronic disease and all-cause mortality would be important to clarify to provide more detailed and consistent dietary recommendations with regard to the amount of whole grains that should be consumed to reduce the risk of chronic disease and premature mortality. Answering this question would also clarify whether there are additional benefits with very high intakes such as those recommended in the Scandinavian guidelines,(35) and whether such high recommendations are justified.”

A list of the confounding factors adjusted for in the included studies were provided in Supplementary Table 3-12. We did mention confounding in the discussion as there were about ten lines of text that described this issue: “Subjects with a high intake of whole grains may have different lifestyles, diets (13;83) or socio-economic status (83) than those with a low intake, thus confounding by other lifestyle factors is a potential source
of bias. In subgroup analyses we found that the associations observed persisted among studies with adjustment for smoking, alcohol, physical activity, BMI and other dietary factors such as sugar-sweetened beverages, red meat, and fruit and vegetables. Differences in socio-economic factors or deprivation could also have influenced the findings, however, both the Nurses’ Health Study and the Health Professionals Follow-up Study, cohorts where there would be relatively little confounding by socio-economic status or deprivation, found similar results to the overall analysis and there was no evidence of heterogeneity in the results when stratified by adjustment for education.

- Another editor felt that it was not clear what the study adds. She also felt that the messaging needs to reflect that effect sizes may differ in other populations. She added that, considering that the variety of whole grains and food preparations is immense, it might be helpful if the introduction talks about the variety of grains or food preparations that fall under refined and whole grain categories.

RESPONSE: We have tried to make this clear and added to the beginning of the discussion: “To our knowledge this is the first comprehensive dose-response meta-analysis that has summarized the association between whole grain intake and several different major chronic disease outcomes, including coronary heart disease, stroke, cardiovascular disease overall, total cancer, all-cause mortality as well as less common causes of death such as mortality from respiratory disease, diabetes, infectious disease, nervous system disorders and all non-cardiovascular, non-cancer causes of death, in a consistent manner.”

We agree that it is possible that the effect sizes may differ in other populations and added to the end of the second paragraph of the discussion: “Given that whole grain consumption differs substantially between populations both with regard to type and amount and because most of the current data is from American and European studies it is possible that effect sizes may differ in other populations.”

We added a sentence about the main sources of whole grains in different areas to the end of the first paragraph: “Consumption of whole grains differs considerably between populations (7) with the main source being whole grain bread in Scandinavian countries (8), whole grain bread and breakfast cereals in the US (9), brown rice, unrefined maize and sorghum in some African countries (10), and brown rice in Asia,(11) although most of the rice consumed in Asia is white rice.(12;13)”

- Another editor felt that the paper lacks relation to a real world setting. He considered that very few people probably eat 3 portions of whole grain, which is what was assessed in the study. He considered that these whole grain eaters may be rather different in many ways than the general population. He was also not fully convinced of the relevance of this paper for general readers.

RESPONSE: We disagree with the first sentence as grains are one of the major staple foods globally. We added to the introduction: “Grains are one of the major staple foods consumed around the world and provide 56% of the energy and 50% of the protein consumed by humans worldwide, and grains constitute the largest component of recommended daily intake in all dietary guidelines.(2) Because of their important role in most diets around the world, health effects of grain consumption, and in particular whole grains is increasing.(3;4) A high intake of whole grains has been associated with reduced risk of type 2 diabetes,(5) coronary heart disease,(6) and obesity.(6)”
We agree that relatively few people eat 3 portions of whole grains, but we think it’s not uncommon to have a total grain intake of 3 portions of more per day, and by replacing refined grains with whole grains it may be achievable to increase whole grain intake considerably. We added to the end of the first paragraph of the discussion: “Relatively few people may have a whole grain intake of 3 servings per day or higher, however, as indicated by the nonlinear dose-response analysis benefits were observed at an intake of even 1 or 2 servings per day in relation to most of the outcomes, thus even moderate increases in whole grain intake could reduce the risk of premature mortality. In addition, a large part of the population may have a total grain intake of 3 servings per day or more, thus replacing most or all of the refined grains consumed with whole grains could increase whole grain intake substantially.”

We agree that whole grain eaters may have different lifestyles than people with a low whole grain intake. Nevertheless, we found that the associations observed persisted in numerous subgroup analyses with adjustment for a range of potential confounders. Potential confounding was mentioned already in the discussion: “Subjects with a high intake of whole grains may have different lifestyles, diets (13;83) or socio-economic status (83) than those with a low intake, thus confounding by other lifestyle factors is a potential source of bias. In subgroup analyses we found that the associations observed persisted among studies with adjustment for smoking, alcohol, physical activity, BMI and other dietary factors such as sugar-sweetened beverages, red meat, and fruit and vegetables. Differences in socio-economic factors or deprivation could also have influenced the findings, however, both the Nurses’ Health Study and the Health Professionals Follow-up Study, cohorts where there would be relatively little confounding by socio-economic status or deprivation, found similar results to the overall analysis and there was no evidence of heterogeneity in the results when stratified by adjustment for education.”

- Another editor felt that the paper needs to more clearly focus on the research question and what is new. She highlighted that you should also make the clinical/how this feeds into dietary advice content easier to understand.

RESPONSE: We added to the discussion: “To our knowledge this is the first comprehensive dose-response meta-analysis that has summarized the association between whole grain intake and several different major chronic disease outcomes, including coronary heart disease, stroke, cardiovascular disease overall, total cancer, all-cause mortality as well as less common causes of death such as mortality from respiratory disease, diabetes, infectious disease, nervous system disorders and all non-cardiovascular, non-cancer causes of death, in a consistent manner.”

We added to the second last paragraph of the discussion: “From a practical angle a whole grain product intake of 120 g/d can be achieved for example by eating a portion of whole grain breakfast cereals (30-40 g) at breakfast, one slice of bread for lunch (30 g) and a piece of whole grain pita bread for dinner (60 g).”
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:

BMJ review

Manuscript ID BMJ.2015.029745 entitled "Whole grain consumption and the risk of cardiovascular disease, cancer, and all-cause mortality – a systematic review and dose-response meta-analysis of prospective studies"

Thank you for the opportunity to review this very relevant meta-analysis on whole grains and risk/mortality of several chronic diseases. I believe that the overall aim of the study is highly relevant, and that BMJ is a very suitable journal for publishing such a paper on a topic which could have a very broad public health impact.

RESPONSE: Thank you very much for the encouraging comment.

I have the following major and minor comments for the authors to consider.

Major:
Overall, the methodological quality of the study is very high, and the PRISMA checklist is followed in a systematic way. However, the presentation of the results is a bit unstructured and the inclusion criteria (which diseases under study, incidence or mortality) and overall aim is not completely clear.

RESPONSE: We have made some corrections in the manuscript to try to clarify this.

1. Endpoints
   - Abstract: In the “study selection” part it is stated that prospective studies of whole grains in relation to cardiovascular disease, total cancer and all-cause mortality are included. In the “results” part, there is reported on cardiovascular disease, total cancer, all-cause mortality AND respiratory diseases, infectious diseases and diabetes. Why this discrepancy?
   
   RESPONSE: There was an error in the abstract and we have added to the sentence “or cause-specific mortality” so it reads: Prospective studies that reported adjusted relative risk estimates for the association between intake of whole grains or specific types of grains and cardiovascular disease, total cancer, all-cause or cause-specific mortality were included. We also modified the title and added: “and cause-specific” so it reads: Whole grain consumption and the risk of cardiovascular disease, cancer, and all-cause and cause-specific mortality – a systematic review and dose-response meta-analysis of prospective studies.

   - It is, thought out the manuscript, not clear when RISK (incidence) and MORTALITY is investigated. This needs to be very clearly stated, also in the presented Figures.

   RESPONSE: For coronary heart disease, stroke, total cardiovascular disease and total
cancer the outcome was either incidence or mortality from these diseases. Subgroup analyses by whether the outcome was incidence or mortality were provided in Supplementary Table 15 and 16. We added to the second sentence of the methods section “incidence or mortality from” so it reads: “Prospective studies of grain intake and incidence or mortality from coronary heart disease, stroke, cardiovascular disease, total cancer, overall mortality and other causes of death were included if they reported adjusted relative risk (RR) estimates and 95% confidence intervals (CIs) and for the dose-response analyses a quantitative measure of the intake for at least 3 categories of grain intake had to be available.”

- In the flow chart, it would be very helpful if an extra level in the bottom could be added with information about how many studies (and publications) are available for each disease endpoint, and whether the endpoint is incidence or mortality.

RESPONSE: We have added this to the end of the flow-chart.

2. Whole-grain assessment
- Whole-grain intake is given as whole-grain products rather than as g/day. It is recommended to use g/day because this eases comparability between studies. It might be that it is not possible to calculate the intake in g/day in this meta-analysis since many of the study simply state the intake in whole-grain products/day. It is, however, important that this is mentioned and discussed. See this reference for more information: Ross, A. B., et al. (2015). "Recommendations for reporting whole-grain intake in observational and intervention studies." Am J Clin Nutr. 2015 May;101(5):903-7.

RESPONSE: We agree and have added to the discussion: “In addition, given the diversity of whole grain products available it is difficult to assess whole grain intake accurately in epidemiological studies and some degree of measurement error is inevitable. A recent review recommended reporting whole grain intakes as the actual amount of whole grain intake per dry weight (86). Since some studies have classified some whole grain items (breakfast cereals, muesli) as whole grain foods if they have a whole grain content of ≥25% or >50% of the weight of the product then a grain product could be considered whole grain if its whole grain content varied between 25-100 or 51-100 grams per 100 grams of the product. Somebody could consume a product with 24 grams or 50 grams of whole grain per 100 grams of the product and still be considered to eat no whole grain, leading to misclassification of the exposure. Most of the studies appeared to report whole grain food intake as the amount or frequency of whole grain food or product intake (fresh weight including water content), while only two publications (10;13) reported intakes in actual amount of whole grain food (dry weight) (10). However, one study which reported results for both whole grain products (fresh weight) and actual whole grain intake (dry weight) in relation to mortality found similar associations for the two (10).”

- 7-7.5 servings is set to the recommended level. It is not clear how and why this cut-off is chosen?

RESPONSE: This was the level at which the greatest reduction in risk was observed for
all-cause mortality as well as several of the other outcomes. We agree that this is a high intake (=210 g/d), but still achievable as shown by the studies in the Scandinavian cohorts. For this reason we also calculated attributable risks for a more modest intake of 4 servings per day (120 g/d). In the revised version of the manuscript we presented the PAR data per 4 servings per day.

- In Scandinavia, the recommended level is 75 g/dag, which roughly is equal up to 250 g whole-grain products. In the Scandinavian EPIC cohorts, where the whole-grain intake is among this highest in observational studies, only participants in the highest quartile comply with this recommendation. (ref. Kyro, C., et al. (2012). "Intake of whole grain in Scandinavia: intake, sources and compliance with new national recommendations." Scand.J.Public Health 40(1): 76-84.). In other cohort studies, very few comply with the recommendation. Thus, it can be relevant to discuss whether it is from both a statistical and a public health viewpoint relevant to calculate the “population attributable risk (PAR)” based on this recommendation. It may be relevant to calculate what impact it will have if people just consumed half of the recommended level. PAR could be shown for both 7 servings per day and for 3,5 servings per day.

RESPONSE: We agree and for this reason we also calculated the PAR for 4 servings per day which was provided in Supplementary Table 17. Because of this we have changed the focus from the PAR per 7 servings per day to 4 servings per day as the reference.

- It is assumed that the proportion of brown rice is 25% of total rice consumed. Even though a reference is added on this, it is not completely clear how the authors came to the conclusion that 25% of the rice consumption must be brown rice?

RESPONSE: In table 1 of the publication from the Nurses’ Health Studies and the Health Professional’s Follow-up study the mean servings per day is provided for white rice stratified by frequency of brown rice intake and vice versa. Therefore we calculated a weighted average of white rice intake weighted by the number of subjects in each category of brown rice intake and of brown rice weighted by the number of subjects in each category of white rice. Then added them together and found the proportion of total rice contributed by white rice and brown rice which was approximately 25% brown rice and 75% white rice. We calculated a weighted average of these amounts which was 167.25 g/d.

Minor:  
- In the results section, it is mentioned that the strongest reduction is observed in at lower whole-grain intakes. Is this due to statistical power, since the majority of the participants in observational studies are in this range? Or do you believe that it has a biological plausible explanation? If the latter is this case, this can be mentioned more in the discussion. From a public health point of view it would then have a great impact, if especially people with a very low whole-grain intake increased their intake. This group might especially
be a difficult group to reach with nutritional campaigns etc.

RESPONSE: We are not sure if there is an underlying biological reason for the observed statistical nonlinearity. As most of the studies had a maximum intake of around 3 servings per day it seems that it might not entirely be an artefact of different studies covering different parts of the dose-response curve. However, we think it is important not to over-interpret the nonlinearity as we are not aware of a mechanism that could explain the nonlinearity of the association and because there is a clear dose-response relationship with larger reductions in risk with higher intakes for most of the outcomes assessed.

We added to the second last paragraph: “The nonlinear analyses suggested that the reductions in mortality risk is steepest at the lowest level of whole grain intake (from 0 to 2 servings/day) and that perhaps targeting subjects with a very low intake might have a greater impact, however, further reductions were observed up to 7-7.5 servings per day or (210-225 g/d), suggesting further benefits with even higher intakes.”

- Page 10, bottom. “and checked by (DCG) for accuracy”. Who is DCG? Is it the co-author Darren Greenwood? It is not completely clear from the abbreviation.

RESPONSE: Yes, that is correct.

I would be happy to review the revised manuscript.

Additional Questions:
Please enter your name: Anne Tjønneland

Job Title: Research Leader

Institution: Danish Cancer Society Research Center
Reviewer: 2

Recommendation:

Comments:
This is an excellent and comprehensive meta-analysis of whole grain intake and risk of CVD, cancer, and all-cause mortality. I have the following minor comments.
RESPONSE: Thank you very much for the encouraging comment.

1. How comparable of the serving sizes across studies? The authors used 3 servings per day of whole grain intake (about median across studies) as the basis for their main results. As a reader, it is hard to link 3 servings/d to actual amounts of, for example, bread or bowl of breakfast cereal. Please make this serving size more understandable in the abstract.
RESPONSE: Not all studies provided a serving size so we are not able to answer the first question. We agree and have added “(90 g = 3 servings and is equal to 2 slices of whole grain bread and 1 bowl of whole grain cereals or 1.5 slice of whole grain pita bread)” so the sentence reads: “The summary RR per 90 g/d increase in the intake (90 g = 3 servings and is equal to 2 slices of whole grain bread and 1 bowl of whole grain cereals or 1.5 slice of whole grain pita bread) ....”

2. The premature death estimates were based on if whole grain intake was increased to 7 servings per day. Would the authors please provide more information on this? I basically would like to know whether this amount is realistic.
RESPONSE: We agree that this level of intake might be challenging to achieve and therefore also presented results of the population attributable fraction according to an intake of 4 servings per day. In the revised version of the manuscript we have presented the PAR data for 4 servings per day.

3. What is the rationale for choosing 3 knots for the splines analyses? Often times based on my personal experience, the shape might have changed dramatically if using different number of knots. How robust were the results presented in this paper?
RESPONSE: The rationale is that the more knots one is using the larger number of categories of whole grain intake are needed to be presented in the individual studies. So to be able to include the largest number of studies in the nonlinear dose-response analysis we used 3 knots.

4. Why excluding one study at a time for the influence analyses. This seems too exploratory to me, and better to have a priori hypothesis for such sensitivity analyses. For example, which dietary assessment tools used to measure grain intake and associated validation studies are important for many studies conducted worldwide.
RESPONSE: Excluding one study at a time is a standard sensitivity analysis that is used to investigate the robustness of the findings. It was not used to investigate a specific
source of heterogeneity, but rather to see whether the associations for the different outcomes were driven by one particularly large study or a study with an outlying result.

We added an analysis by whether the dietary assessment was validated to Supplementary Table 15 and 16 and added a sentence to the section on subgroup analyses: “In the analysis of whole grain intake and stroke there was marginally significant heterogeneity by whether the dietary assessment method had been validated, $p_{\text{heterogeneity}}=0.06$, with a significant association among studies which used a validated method, but not in the one study that didn’t, however, there was no heterogeneity in this subgroup for the other outcomes (Supplementary Table 15 and 16).”

Additional Questions:
Please enter your name: Xuehong Zhang

Job Title: Instructor

Institution: Harvard Medical School and Brigham and Women's hospital
Reviewer: 3

Recommendation:

Comments:
I find this review about whole grains in relation to risk/mortality of several major diseases of high relevance. I further think that this paper is highly suitable for BMJ, because it is important that e.g. general practitioners and other health professionals are aware of the role of whole grains in prevention of especially non-communicable diseases.

RESPONSE: Thank you very much for the encouraging comment.

The study is of very high analytical quality in relation to the applied methods. I, however, have concerns regarding the identification of included studies, and I also think that difficulties in whole-grain assessment needs to be addressed further. Below please find detailed comments:

RESPONSE: We have added additional details regarding the identification of included studies as well as the assessment of whole grain intake. Please see the following responses below.

Major comments:
- It is unclear to me when incidence or mortality is investigated? And in all studies of both incidence and mortality are included, or is it a priori is decided to focus on specially mortality. If so, why are incidence studies also included. I think that it would be fair to include both, but it is of outmost importance that it clearly is described in the text and tables/figures, whether the study is on incidence or mortality.

RESPONSE: For coronary heart disease, stroke, total cardiovascular disease and total cancer the outcome was either incidence or mortality from these diseases. Because the number of studies is limited, analyses restricted to only incidence or mortality were less meaningful and therefore in the figures we have not stratified by the outcome subtype (we would prefer not to stratify the analysis in the figures), however, we also provided analyses stratified by whether the outcome was incidence or mortality of these diseases in Supplementary Table 15 and 16. In the main analysis we presented results overall for both outcome types combined, but also conducted subgroup analyses by whether the outcome was incidence or mortality and these were provided in Supplementary Table 15 and 16. Importantly there was no significant heterogeneity between studies that reported on incidence vs. mortality from these outcomes. We have tried to make this clearer in the manuscript. We modified the 2nd sentence of the methods so it reads: “Prospective studies of grain intake and incidence or mortality from coronary heart disease, stroke, cardiovascular disease, total cancer, and all-cause and cause-specific mortality….”
In is not clear which diseases that it a priori was decided to include. In the abstract for instance, T2D is not mentioned in the study selection, but T2D is investigated. This needs to be more clear.

RESPONSE: We included a priori any study reporting on CHD, stroke, CVD, total cancer, all-cause or cause-specific mortality. Because several of the studies on all-cause mortality also reported on cause-specific mortality studies on mortality from infectious, neurological, and respiratory disease, and diabetes mortality as well as all non-cardiovascular, non-cancer causes of death were included in the analysis. We made a correction to the abstract and added “or cause-specific mortality” so the sentence reads: “Prospective studies that reported adjusted relative risk estimates for the association between intake of whole grains or specific types of grains and cardiovascular disease, total cancer, all-cause or cause-specific mortality were included.”

Whole-grain assessment: It is important that difficulties in assessing the whole-grain intake is discussed including the reason for not reporting the whole-grain intake in g/day.

RESPONSE: We have re-calculated the intakes to grams per day of whole grain products for comparison with our previous meta-analysis on whole grains and colorectal cancer risk. However, because of the limited number of studies that reported on absolute whole grain intake (dry weight) compared to whole grain products (fresh weight) analyses were conducted for whole grain products rather than absolute whole grain intake.

We added to the discussion the following: “In addition, given the diversity of whole grain products available it is difficult to assess whole grain intake accurately in epidemiological studies and some degree of measurement error is inevitable. A recent review recommended reporting whole grain intakes as the actual amount of whole grain intake per dry weight (93). Since some studies have classified some whole grain items (breakfast cereals, muesli) as whole grain foods if they have a whole grain content of ≥25% or >50% of the weight of the product then a grain product could be considered whole grain if its whole grain content varied between 25-100 or 51-100 grams per 100 grams of the product. Somebody could consume a product with 24 grams or 50 grams of whole grain per 100 grams of the product and still be considered to eat no whole grain, leading to misclassification of the exposure. Most of the studies appeared to report whole grain food intake as the amount or frequency of whole grain food or product intake (fresh weight including water content), while only two publications (8;19) reported intakes in actual amount of whole grain food (dry weight) (8). However, one study which reported results for both whole grain products (fresh weight) and actual whole grain intake (dry weight) in relation to mortality found similar associations for the two (8).”

It needs to be addressed why seven servings were set as optimal reference. Please add a reference and argue for choosing this cut-off. For instance, in Norway, Sweden and Denmark the recommendation is 75 g/day. These Scandinavian recommendations could be mentioned.

RESPONSE: We used 7 servings per day as optimal as it was the level of intake that gave the highest reduction in risk. We agree that this is a quite high intake and for this reason we also presented population attributable fractions based on a more modest
reference intake of 4 servings per day as well. In the revised manuscript we therefore have focused the PAR on an intake of 4 servings per day (120 g/d).

We added the following to the introduction to address the differing dietary recommendations: “In spite of a growing body of epidemiological evidence for the health benefits of whole grain consumption, dietary recommendations have often been unclear or inconsistent with regard to the amount of whole grains that should be eaten to reduce chronic disease risk. For example in the World Cancer Research Fund 2007 report it was recommended individuals should "eat relatively unprocessed cereals (grains) and/or pulses with every meal",(25) while in the United Kingdom there is no specific recommendation other than "choosing whole grain, brown or high fibre varieties wherever you can", but no specific quantities of whole grains were recommended.(26) In the USA and Canada the recommendation is that "all adults eat at least half their grains as whole grains" so at least 3 servings of whole grains should be consumed per day,(27) while in the Scandinavian countries at least 75 grams per day of whole grain intake (dry weight) which equals approximately 250 grams per day (~8 servings/day) of whole grain products (fresh weight) is recommended (28). There may be several reasons for the inconsistent dietary guidelines for whole grain intake including difficulties in measuring whole grain intake, differences in the consumption patterns of whole grains between populations, or lack of data on whole grain intake in some populations, but they may also be because most previous meta-analyses only considered selected disease endpoints and did not conduct dose-response analyses.(5;24) Some of the current authors found a reduced risk of type 2 diabetes incidence with up to 2-3 servings per day (60-90 g/d) of whole grain intake, but no further reductions in risk with higher intakes,(4) while in a second meta-analysis of whole grain intake and colorectal cancer a linear inverse association was observed up to an intake of 180 g/d.(21) Whether the association is linear or reaches a plateau for other chronic disease outcomes and all-cause mortality, or whether only specific types of whole grains are associated with chronic disease and all-cause mortality would be important to clarify to provide more detailed and consistent dietary recommendations with regard to the amount of whole grains that should be consumed to reduce the risk of chronic disease and premature mortality. Answering this question would also clarify whether there are additional benefits with very high intakes such as those recommended in the Scandinavian guidelines,(28) and whether such high recommendations are justified.

If the above mentioned comments are addressed, I definitely think that the study would be suitable for publication in BMJ.

Additional Questions:
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