Author's response to reviews

Title: Dietary calcium intake and mortality risk from cardiovascular disease and all causes: a meta-analysis of prospective cohort studies

Authors:

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Version: 2
Date: 20 June 2014

Author's response to reviews: see over
June 20, 2014
Joanna Denyer, PhD
Senior Assistant Editor
BMC Medicine

RE: MS 1271514873127659
Title: “Dietary calcium intake and risk of mortality from cardiovascular disease and all causes: a meta-analysis of prospective cohort studies”
Authors: Xia Wang, Hongxia Chen, Yingying Ouyang, Jun Liu, Gang Zhao, Wei Bao, and Maosheng Yan

Dear Dr. Denyer,

Please find the revised MARKED manuscript with changes highlighted and the revised CLEAN manuscript attached. Please also see below for a response to the Editor’s comments and a point-by-point response to the reviewers’ comments.

Thank you for carefully reading the manuscript and providing positive comments. To address the reviewers’ comments, we consulted more recent literature on this topic and carefully revised the manuscript as the reviewers suggested.

We are very grateful to the reviewers for their helpful suggestions. We are pleased that the reviewers agree that the manuscript will be a valuable contribution to the literature in this area. We feel that the quality of the manuscript has been significantly improved as a result of the revisions. We hope that the revised manuscript is suitable for publication in *BMC Medicine*. However, if further changes are needed, please feel free to contact me.

Thank you for your kind consideration.

Kind regards,
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**Response to the reviewers’ comments:**

**Reviewer: 1**

**Comments:**

Abstract: The description of the U-shaped relation of dietary calcium in the results section does not agree with Figure 3. 800 mg/d was the minimum, with increased mortality rates for both lower and higher intakes, albeit not statistically significant for lower intakes. In addition, the calcium intake-CVD relation is a true U-shaped curve while that for all-cause mortality rate is not. Suggest that the two relations be described separately. There are, for example, beneficial effects of calcium intake on risk of some cancers such as colorectal cancer.

**Response:**

Thank you for your careful review of our manuscript. As the reviewer suggested, we have carefully revised the manuscript. The relation of dietary calcium and all-cause and cardiovascular mortality has been described separately. Text has been revised to incorporate reviewers comment.

For details, please refer to the point-by-point response to the comments and suggestions of the reviewers (below).

**Comments:**

This paper should be cited

**Response:**

We agree with the comments. As the reviewer suggested, we have carefully revised the manuscript. The study by Paik et al. has be cited in the revised version of the manuscript.

**Comments:**

Discretionary Revisions
In the studies cited, when was calcium intake assessed? If at the time of enrollment, what guarantee is there that the same intake persisted over the duration of the study? There could be changes during the study. There should be a section on strengths and limitations of this meta-analysis and concern about constancy of calcium intake could be one of the limitations.

**Response:**

Thanks for the reviewer’s comments. We agree with the comments and, as the reviewer suggested, we have carefully revised the limitation section of the manuscript. Dietary calcium intake was measured at baseline in the included studies. During the long follow-up, participants may have changed their diets. Therefore, we were not able to evaluate change in dietary calcium intake during follow-up.
Comments:
While some of the studies included consideration of vitamin D, vitamin D was not discussed in the text. I think it is important to do so. Serum 25-hydroxyvitamin D levels are inversely correlated with cardiovascular disease incidence rates [Anderson, 2010; Wang, 2012]. Can the studies be separated into those that considered vitamin D and those that did not?


Response:
Thanks for the reviewer’s comments. We agree with the comments and, as the reviewer suggested, we have carefully revised the manuscript. We performed subgroup analyses by whether or not a study adjusted for vitamin D status. In those studies which adjusted for vitamin D status, dietary calcium intake were not significantly associated with cardiovascular mortality (RR 0.92; 95% CI, 0.74, 1.15; \( P = 0.479 \); Table 2)).

For all-cause mortality, after adjusting for vitamin D, there remained no significant association between dietary calcium intake and all-cause mortality (RR 0.87; 95% CI, 0.65, 1.16; \( P = 0.332 \); Table 2)).

Comments:
Magnesium counters the effects of calcium on risk of cardiovascular disease. Did any of the studies consider dietary magnesium? It would be worthwhile to discuss the calcium-magnesium balance in the risk of cardiovascular disease.

Response:
Thanks for the reviewer’s comments. As the reviewer suggested, we have carefully revised the discussion section. Heterogeneity could also be caused by differences in dietary magnesium intake. In the included studies, no study adjusted for dietary magnesium intake. However, some studies showed that dietary magnesium intake was associated with reduced mortality from cardiovascular disease (1, 2). It is possible that magnesium counters the effects of calcium on risk of cardiovascular disease. Furthermore, the result of several studies also suggests that balance between calcium and magnesium can be necessary for the prevention of cardiovascular disease (3, 4). Text has been revised to incorporate reviewers comment.

Comments:
What was the effect of sex and age on the risk of cardiovascular disease and mortality rates with respect to dietary calcium?

Response:
Thanks for the reviewer’s comments. As the reviewer suggested, we have carefully revised the discussion section. Sex and age have effect on the risk of cardiovascular disease and mortality rates with respect to dietary calcium. Supplemental calcium intake was associated with an elevated CVD mortality in men but not in women (5). Dietary supplement use is more common in women than in men (6). Male users of calcium supplements may start taking calcium supplements at an older age.

Comments:
Additional papers to consider citing:

Response:
We agree with the comments. As the reviewer suggested, we have carefully revised the manuscript. These studies have be cited in the revised version of the manuscript.
Reviewer: 2
Comments:
Figure 2 is a lovely graph, but there are a number of things that appear very wrong with it. The authors state that the reference intake is 800g/day, so the relative risk at 800g/day should be 1, because everything is relative to that intake. But *all* relative risks are above 1. That cannot be correct. Furthermore, at the reference intake, the standard errors should be zero, because the relative risk is fixed as 1.0. But the confidence intervals on the graph do not reflect this. I can only conclude that there is something wrong with either the plotting of the graph, or worse the model on which it is based. The graph for all-cause mortality in the supplement seems have done this better, so it is worrying that CVD mortality does not. The authors need to look into this.

Response:
Thank you for your careful review of our manuscript. Figure 3 has been modified in the revised version according to suggestions from the reviewer. Text has been revised to incorporate reviewers comment. Detailed revisions are listed below.

Comments:
Results for men and women from the same study are included in the meta-analysis as if they were from separate studies. This leads to under-estimation of the heterogeneity and wrong tests of heterogeneity. Instead, the authors should first combined the results from the men and the women separately using fixed effects, so that each study only has one result, before combining with the other studies as normal.

Response:
We agree with the comments and have addressed these points in the revised version. Results for men and women from the same study have been combined using fixed effects prior to inclusion in the overall analyses. Accordingly, all forrest plots have been modified in the revised version.

Comments:
There is some inconsistency in the reporting of heterogeneity. Some meta-analyses have it, some don’t. But more importantly, there is no exploration of this heterogeneity. There should be, and this would form a useful addition to the review.

Response:
We agree with the comments and have addressed this point in the revised version. To more clearly state this issue the reviewer raised, some new references were added in the revised version. Also the limitations section in the discussion was restructured in the revised version. Detailed revisions are listed below.

Comments:
A final comment is that investigating CVD mortality a quite different thing from CVD incidence. The introduction and aims suggest that such CVD events are what the authors are really interested in, but have not looked at. Hypothetically you could have different causes.
Response:
Thank you for your careful review of our manuscript. The primary outcomes in this analysis are cardiovascular mortality and all-cause mortality. We have rephrased some sentences in the Introduction section to avoid confusion. We have incorporated the reviewer’s specific comments in preparation of revised version of manuscript.

Detailed revisions are listed below.

Comments:
The axes on the forest plots would benefit from better labelling and numbers on the axes.
Response:
We thank the reviewer for the valuable suggestion. All forest plots have been modified in the revised version.

Comments:
Both CVD mortality and all-cause mortality should ideally use the same reference intake so that we can compare the curves.
Response:
Thanks for the reviewer’s comments. The curves of both CVD mortality and all-cause mortality have been modified in the revised version according to suggestions from the reviewer.

Comments:
It would be helpful to see the levels of intake contributing to the curves indicated on the graph, so the reader can see where the bulk of the data is, and how many studies / observations are in the extremes.
Response:
We thank the reviewer’s comment. We performed a 2-stage random-effects dose-response meta-analysis to examine a potential nonlinear relation between dietary calcium intake and risk of mortality from cardiovascular disease and all causes. This was done by modeling dietary calcium intake using restricted cubic splines with 3 knots at fixed percentiles (10%, 50%, and 90%) of the distribution (7). In the first stage, a restricted cubic spline model with the 2 spline transformations was estimated using generalized least-squares regression taking into account the correlation within each set of published relative risks as described by Orsini et al. (8). In the second stage, we combined the 2 regression coefficients and the variance/covariance matrix that had been estimated within each study, using the restricted maximum likelihood method in a multivariate random-effects meta-analysis. The pooled relative risks for specific exposure values were estimated using a procedure described by Orsini and Greenland (9). A P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero.
In our manuscript, the pooled relative risks for dietary calcium intake were presented using a standard two-way plot instead of our previous method, which aids in the interpretation and presentation of a nonlinear relationship in graphical form. This
approach was implemented in many previous meta-analyses published in Am J Clin Nutr 2013,97(5):951-957 (see study by Larsson et al) (10), Am J Epidemiol 2011;174(9):993-1001 (see study by Larsson and Orsini) (9), Circulation 2014; 129(6): 643-59 (see study by Ding et al) (11), etc.

Comments:
The authors state “A test for a nonlinear relationship was calculated by setting the coefficient of the second spline equal to zero.” Constraining the coefficient to be zero is not of itself a test. Maybe they then compared this to an unconstrained model? Maybe they simply tested the null hypothesis that this coefficient was zero? This should be put more clearly.

Response:
Thanks for the reviewer’s comments.
In this study, a P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero. This approach has been used in previous dose-response meta-analyses, e.g., Larsson and Orsini (Am J Epidemiol. 2011;174(9):993-1001)(9). We have modified this in the revised manuscript.

Comments:
It is not clear whether the dietary calcium intake used as the main exposure includes dietary supplements or not. Isn’t total intake the most relevant exposure? Maybe the authors could be clearer on this in the methods and descriptive tables.

Response:
Thanks for the reviewer’s comments. Text have been modified in the revised version according to suggestions from the reviewer. The primary exposure variable was dietary calcium intake, which was estimated from foods only, but we also examined supplemental calcium. Owing to few studies examining total calcium (from both), we did not estimated total calcium.

Comments:
Calcium absorption may be modified by vitamin D status. Several of the included studies report on this. This would therefore be an important subgroup analysis to explore this potential source of heterogeneity in the results. In the absence of adequate information on vitamin D status, would latitude be a useful proxy?

Response:
Thank you for your careful review of our manuscript. We appreciate the comments and, as the reviewer suggested, we have carefully revised the manuscript. Considering the effect of vitamin D status on calcium absorption, we performed subgroup analyses by whether or not a study adjusted for vitamin D status. In those studies which adjusted for vitamin D status, dietary calcium intake were not significantly associated with cardiovascular mortality (RR 0.92; 95% CI, 0.74, 1.15; \( P = 0.479 \); Table 2). For all-cause mortality, after adjusting for vitamin D, there remained no significant association between dietary calcium intake and all-cause mortality (RR 0.87; 95% CI,
Serum vitamin D level shows a strong correlation with the latitude (12). In the absence of adequate information on vitamin D status, latitude would be a useful proxy. Except during the summer months, the skin makes little if any vitamin D from the sun at latitudes above 37 degrees north (in the United States, the shaded region in the map) or below 37 degrees south of the equator. People who live in these areas are at relatively greater risk for vitamin D deficiency.

**Point-by-Point Response to Reviewers provided below:**

**Title page:**
Page 1, line 1-3: The title was revised to “Dietary calcium intake and mortality risk from cardiovascular disease and all causes: a meta-analysis of prospective cohort studies”
Page 1, line 4-5: The authors were changed to “Xia Wang¹², Hongxia Chen³, Yingying Ouyang², Jun Liu², Gang Zhao⁴, Wei Bao⁵*, and Maosheng Yan⁵*”
Page 1, line 6: Correspondence was changed to “Correspondence: wei.bao@nih.gov; or 254251509@qq.com” and authors details were removed before reference of text.
Page 1, line 7-9: This segment was revised to “²Department of Nutrition and Food Hygiene, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, 13 Hangkong Road, Wuhan 430030, China”
Page 1, line 10-12: This segment was changed to “⁵Guangdong Provincial Key Laboratory of Occupational Disease Prevention and Treatment, Guangdong Prevention Hospital for Occupational Disease Prevention and Treatment, Xingongxi Road 68, Guangzhou 510300, China”
Page 1, line 13: Change “Main text 2564” to “Main text 3268”
Page 1, line 15: Delete “Disclosures: The authors have no conflicts of interest to declare in relationship to this manuscript.”

**Abstract**
All P value were changed to italic capitals in the text.
Page 2, line 6-7: The sentence “Relevant studies were identified by searching……Library databases until 30 December 2013.” was changed to “We identified relevant studies by searching……Library databases between 1 September 2013 and 30 December 2013.”
Page 2, line 10: Change “…all-cause and cardiovascular mortality were eligible.” to “…cardiovascular and all-cause mortality were eligible.”
Page 2, line 13: Delete “some”
Page 2, line 17: Delete “and all-cause”
Page 2, line 17: The sentence was revised to “Intakes that were lower and higher than 800 mg/day were gradually associated with a higher risk of cardiovascular mortality.”
Page 2, line 18-19: Insert the sentence “For all-cause mortality, we also observed a threshold effect between about 900 and 1100 mg/day.”
Page 2, line 20: The sentence was revised to “The risk of all-cause mortality did not decrease further at intakes above 900 mg/day.”
Page 2, line 22-23: Change “…cardiovascular and all-cause mortality in a U-shape manner.” to “…cardiovascular mortality in a U-shaped manner and that high dietary calcium intake (> 900 mg/day) was not associated with a decreased rate of all-cause
mortality.”

Page 3, line 1: Key Words were revised to “Calcium, Cardiovascular disease, Cohort, Meta-analysis, Mortality”

**Background**

Page 3, line 3: Change “mineral” to “minerals”
Page 3, line 4: The sentence was revised to “The body tightly controls circulating levels of calcium, usually maintaining a constant range of 1.0–1.2mmol/L.”
Page 3, line 6: Change “encouraged” to “recommended by many health care professionals”
Page 3, line 7: Insert the word “Consequently,”
Page 3, line 9: The sentence was revised to “The health effects of calcium intake on nonskeletal outcomes, including cardiovascular mortality and all-cause mortality, have received growing attention.”
Page 3, line 12: Change the word “ischaemic” to “ischemic”
Page 3, line 13: Change the word “randomised” to “randomized”
Page 3, line 15: Insert the segment “which are two major causes of cardiovascular mortality”
Page 3, line 16: Change “cardiovascular events or all-cause mortality” to “cardiovascular and all-cause mortality”
Page 3, line 17-18: The sentence was revised to “Differences in calcium intake doses, which were higher in the trials, may lead to the differences ……”

**Methods**

Page 4, line 5: Change “September 2013” to “1 September 2013”
Page 4, line 10: Insert the word “and”
Page 4, line 11: The sentence was revised to “No language restrictions were imposed on publications. Furthermore, we identified additional articles by manually searching the reference lists of pertinent articles and recent reviews.”
Page 4, line 18-20: The sentence was revised to “We excluded studies with ecological, case-control, or cross-sectional designs; studies with no adjustment for potential confounders; and studies that did not report RRIs or hazard ratios and corresponding 95% CIs.”
Page 4, line 23-24: Insert the segment “which was estimated from foods only, but we also examined supplemental calcium”
Page 4, line 25: Insert “mortality from” before “all causes”
Page 5, line 3-4: Change “The following characteristics of the identified papers were recorded” to “We recorded the following characteristics for each identified paper”
Page 5, line 10: The sentence was revised to “The system allowed a total score of 0–9 points (9 represented the highest quality).”
Page 5, line 14: Insert “we used”
Page 5, line 15: Delete “was used”
Page 5, line 16: Delete “to be”
Page 5, line 18: Change “Data Synthesis and Analysis” to “Data synthesis and analysis”
Page 5, line 23: Change “were” to “was”
Page 6, line 8-10: The sentence was revised to “A P value for a nonlinear relationship
was calculated by testing the null hypothesis that the coefficient of the second spline was equal to zero.”

**Results**

Page 6, line 16: Change “exclusion of” to “excluding”
Page 6, line 19: Change “due to reporting” to “because they reported”
Page 7, line 1: Change “total CVD” to “total cardiovascular disease (CVD)”
Page 7, line 3: Delete “had”
Page 7, line 8-9: The sentence was revised to “One study used one week food frequency recall to measure dietary calcium intake, and all other studies used food frequency questionnaires.”
Page 7, line 11: Change “BMI” to “body mass index”
Page 7, line 13: Delete “but overall the level was adequate”
Page 7, line 17: Delete “a total of”
Page 7, line 20-22: The sentence was revised to “……the highest and lowest level of dietary calcium intake was 0.97 (95%CI: 0.89 to 1.07; \( P = 0.60 \)), with no heterogeneity among the studies (\( I^2 = 18.8\%; P = 0.28 \)) (Figure 2).”
Page 7, line 24: Change “Begg, \( p = 0.28 \); Egger, \( p = 0.31 \)” to “Begg, \( P = 0.75 \); Egger, \( P = 0.11 \)”
Page 7, line 25: The sentence was revised to “The dose-response analysis revealed evidence of a nonlinear association (\( P\)-nonlinearity < 0.01).”
Page 8, line 3: Change “figure 3” to “Figure 3”
Page 8, line 7-11: The fragment was revised to “Compared to individuals with 500 mg/day of dietary calcium intake, the predicted RR for cardiovascular mortality were 0.95 (95% CI: 0.87 to 1.03) for individuals with 800 mg/day of calcium intake, 0.97 (95% CI:0.89 to 1.05) for 1000 mg/day, 1.01 (95% CI: 0.94 to 1.08) for 1200 mg/day, and 1.06 (95% CI: 0.99 to 1.14) for 1400 mg/day.”
Page 8, line 14-24: The fragment was revised to “One study was not included in our analysis, because it reported only one intake level of calcium. The summary relative risks of all-cause mortality comparing the highest and lowest level of dietary calcium intake was 0.83 (95%CI: 0.70 to 1.00; \( P = 0.05 \)), with significant heterogeneity among the studies (\( I^2 = 74.9\%; P = 0.003 \)) (see Additional file 2: Figure S1). No significant publication bias was observed (Begg test, \( P = 0.46 \); Egger test, \( P = 0.34 \)).

We found evidence of a nonlinear association (\( P\)-nonlinearity < 0.01) (see Additional file 2: Figure S2). We found a threshold effect between about 900 and 1100 mg/day. Compared to intakes 900 mg/day, a lower intake was gradually associated with a higher risk of all-cause mortality. At intakes above 900 mg/day, risk of all-cause mortality did not reduced with the increase of dietary calcium intake.”

Page 9, line 5: Change “…any supplements (RR, 1.00; 95% CI 0.86 to 1.15; \( p = 0.96 \)) (Additional Figure S3).” to “… any supplements (RR = 0.96; 95% CI 0.82 to 1.13; \( P = 0.66 \)) (see Additional file 2: Figure S3).”
Page 9, line 7: Change “Sensitivity analysis” to “Subgroup and sensitivity analyses”
Page 9, line 8-13: The fragment was revised to “To test the robustness of the results and investigate the sources of between-study heterogeneity, we conducted subgroup
analyses. Table 2 presents the different subgroup analyses of studies on cardiovascular and all-cause mortality. The associations between dietary calcium intake and risk of cardiovascular and all-cause mortality did not differ substantially by study location, sex, or whether vitamin D status were controlled for in models.”

Page 9, line 14-16: Insert the part “For all-cause mortality, subgroup analyses showed that dietary calcium intakes were not significantly associated with an increased mortality risk in studies with more than 10 years of follow-up period, but not in studies with less than 10 years.”

Page 9, line 17-20: The part was revised to “We also performed a sensitivity analysis. Exclusion of one study that enrolled elderly people aged more than 65 years yielded similar results for cardiovascular mortality (RR = 1.00; 95% CI: 0.94 to 1.07; \( P = 0.90 \)) or all-cause mortality (RR = 0.89; 95% CI: 0.76 to 1.05; \( P = 0.18 \)).”

**Discussion**

Page 9, line 23: Delete “and all-cause”

Page 9, line 23 – Page 10, line 3: The fragment was revised to “Compared with intakes 800 mg/day, both lower and higher intakes were gradually associated with a higher risk of cardiovascular mortality. For all-cause mortality, we also observed a threshold effect between about 900 and 1100 mg/day. Intakes above 900 mg/day was not associated with a decrease in risk of all-cause mortality.”

Page 10, line 4-14: Insert the part “To explore a potential source of heterogeneity in our results, we considered vitamin D status associated with dietary calcium intake. However, subgroup analyses showed that dietary calcium intakes were not significantly associated with all-cause mortality in studies that adjusted for vitamin D status. Vitamin D, directly or indirectly, enhances renal conservation of the absorbed calcium and intestinal absorption of calcium. Some studies have noted that serum 25-hydroxyvitamin D levels, the major circulating metabolite of vitamin D, are inversely correlated with cardiovascular disease incidence rates. The coadministration of calcium with vitamin D may influence the adverse effect of dietary calcium. Therefore, although the sensitivity analysis could not explain the level of heterogeneity, it could also be because of the differences of vitamin D status among the studies.”

Page 10, line 15-21: Insert the part “Heterogeneity could also be caused by differences in dietary magnesium intake. None of all included studies adjusted for dietary magnesium intake. However, some studies showed that dietary magnesium intake was associated with reduced mortality from cardiovascular disease. It is possible that magnesium counters the effects of calcium on risk of cardiovascular disease. Furthermore, some studies also suggests that balance between calcium and magnesium may help prevention cardiovascular disease.”

Page 10, line 22 – Page 11, line 7: Insert the part “Another possible explanation for the differences between the studies might be the effects of sex and age on the risks of cardiovascular disease and mortality rates with respect to dietary calcium or supplemental calcium intake. Supplemental calcium intake was associated with an elevated CVD mortality in men but not in women. A recent study by Paik et al also found that supplement calcium intake did not increase CVD risk in women. Dietary
supplement use is more common in women than in men. Additionally, male users of calcium supplements may start taking calcium supplements at an older age.”

Page 10, line 9: Change the word “ischaemic” to “ischemic”

Page 10, line 16: Delete “and all-cause”

Page 10, line 17-21: The fragment was revised to “…showed that high dietary calcium intake was related to a lower risk of stroke in populations with low to moderate calcium intakes. Therefore, increased dietary calcium intake may be associated with reduced cardiovascular mortality risk at low to moderate calcium intakes.”

Page 11, line 22 – Page 12, line 6: The part was revised to “In addition, our study showed that, in comparison with intakes of 800 mg/day, a higher intake was associated with increased risk of cardiovascular mortality. In a reanalyses of randomised trials, a higher rate of myocardial infarction was observed for calcium supplementation. The results from a meta-analysis of randomised trials of calcium supplements also indicated a higher risk of cardiovascular events in women with higher intake levels of dietary calcium, but not in women with lower intake levels. Thus, it is most likely that increasing dietary calcium intake may increase risk of cardiovascular mortality, in individuals who already consume adequate amounts of calcium.”

Page 12, line 7-14: Insert the part “Our results are concordant with findings from a recent review in which calcium intake was not associated with risk of cardiovascular disease when comparing the highest and lowest intake levels. However, this study did not evaluate the dose-response relation of dietary calcium intake and cardiovascular disease. In the current study, dose-response analysis showed that the association between dietary calcium intake and cardiovascular mortality is U-shaped. The null findings to date for comparing the highest and lowest calcium intake categories likely relate to the nonlinear association.”

Page 12, line 15 – Page 13, line 2: Insert the part “Moreover, a recent meta-analysis showed that higher calcium intake may be associated with a reduction in risk of colorectal cancer beyond 1,000 mg/day. There could be beneficial effects of calcium intake on risk of some cancers such as colorectal cancer. However, a meta-analysis of randomised controlled trials suggested no effect of calcium on the risk of total cancer. In the present study, a high calcium intake (> 900 mg/day) was not associated with a decreased rate of all-cause mortality. For all-cause mortality, subgroup analyses showed that dietary calcium intakes were not significantly associated with an increased mortality risk in studies with more than 10 years of follow-up period, but not in studies with less than 10 years. It is possible that the follow-up periods in these studies were too short to identify the true associations with mortality. In fact, the longer induction periods were observed for cancers than for cardiovascular disease.”

Page 13, line 3-10: Insert the part “For calcium supplement use, a previous review suggested that the evidence regarding the relationship between calcium supplement use and increased cardiovascular disease risk is insufficient. A study by Radford et al showed that the effects of calcium supplements on cardiovascular risk did not differ across varying patient subpopulations, such as younger people and those with low dietary calcium intake. The present study also found no evidence that calcium
supplements increased the risk of cardiovascular mortality. Thus, more longitudinal studies are be needed to confirm or refute the results of our study.”

Page 13, line 11-25: The part was revised to “Calcium is a …… calcitropic hormones. Alterations in calcium homeostasis caused by diets that are low or very high in calcium can alter blood levels of calcium and calcitropic hormones. Calcium intakes that are too low may cause mortality or CVD risk through pathways that affect blood pressure, insulin secretion and sensitivity, and blood cholesterol concentrations. Likewise, excessive amounts of calcium may also exert a harmful effect on cardiovascular health by inducing a hypercoagulable state. Many studies show that high levels of circulating fibroblast growth factor-23 are associated …… may also increase risk of mortality associated with vascular and soft tissue calcification and effects on arterial stiffness.”

Page 14, line 2: Delete the word “have” and change the word “each” to “all”
Page 14, line 4: change the word “helps” to “helped”
Page 14, line 7-14: The fragment was revised to “Moreover, dietary calcium intake was measured at baseline in the included studies. During the long follow-up, participants may have changed their diets, including changing their dietary calcium intakes. Therefore, we were not able to evaluate change in dietary calcium intake during follow-up. Besides, calcium intake levels were assessed by food frequency questionnaires in most studies. Measurement errors of calcium intake were possible. However, the prospective nature of the included studies could have led to an underestimation of the real association and could not explain the positive associations we observed in this study.”

Page 14, line 15-20: The part was revised to “Our results, together with previous studies, suggest that increasing dietary calcium intake was associated with reduced mortality risk at low to moderate calcium intakes, while it was not associated with a decreased risk of mortality at high calcium intakes. Thus, intake recommendations for calcium should consider individual characteristics and should focus on people with low intake levels of calcium, rather than increasing the intake of those with adequate amounts of calcium.”

**Conclusions**
Page 14, line 22-24: The section was revised to “This meta-analysis suggests a U-shaped relationship between dietary calcium intake and risk of cardiovascular mortality. A high calcium intake (> 900 mg/day) was not associated with a decreased rate of all-cause mortality.”

**Additional files**
Page 15, line 4: Delete the word “the”
Page 15, line 8: Change “700” to “500”

**Abbreviations**
Page 15, line 11: Insert “CI: confidence interval; CVD: cardiovascular disease; RR: relative risk.”

**Authors’ contributions**
Page 15, line 17: Insert “MSY”
Page 15, line 19: Insert “HXC, MSY”

**Funding**


