PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Dietary Calcium and Magnesium, Calcium/magnesium Ratio, and Mortality: Results from the Shanghai Women's and Men's Health Studies
AUTHORS	Dai, Qi; Shu, Xiao-Ou; Deng, Xinqing; Xiang, Yong-bing; Li, Honglan; Yang, Gong; Shrubsole, Martha; Ji, Butian; Cai, Hui; Chow, Wong-Ho; Gao, Yu-Tang; Zheng, Wei

VERSION 1 - REVIEW

REVIEWER	Xuehong Zhang, MD, ScD Instructor in Medicine, Harvard Medical School, USA Associate Epidemiologist Brigham and Women's Hospital, USA I have no conflict of interest.
REVIEW RETURNED	15-Oct-2012

THE STUDY	there is no supplementary documents to this ms.
GENERAL COMMENTS	A group of stellar epidemiologists conducted an study in Asian population to test whether Ca/Mg intake ratio modify the associations between intakes of Ca and Mg with all-cause mortality and cause-specific mortality. The topic is interesting and the paper is well written. I have minor constructive comments as follows: 1. The Objectives in the abstract is worded such that the authors tested the hypotheses on mortality due to gastrointestinal tract cancer. But the results mentioned only colorectal cancer and gastric cancer. It is unclear whether the hypothesis only applies to these two cancer sites. If not, it seems worthwhile mentioning the results of other gastrointestinal tract cancers. 2. Given that postmenopausal hormone use may potentially influence CVD or cancer mortality, is information on postmenopausal hormone use available in women? How results look at after adjusting for hormone use in SWHS? 3. Tables already contain comprehensive data. But it might be better to show the number of cases for significant associations when the numbers are relative small for the stratified analysis by ratio (i.e., Table 2, colorectal cancer and gastric cancer) in table footnotes. This will provide some information on how robust the results were.

REVIEWER	Rosanoff, Andrea Center for Magnesium Education & Research
REVIEW RETURNED	12-Nov-2012

THE STUDY	There are no supplemental documents in the submission.
REPORTING & ETHICS	This important study expands the focus of the importance of Ca:Mg intake ratio on the disease preventing aspects of nutritional Ca and nutritional Mg to a population with a low Ca:Mg dietary ratio. Until now, such studies have been limited to populations with a high Ca:Mg dietary ratio. The impact is on important diseases (cardiovascular; cancer) that are rising globally as consumption of the modern process food diet expands.
GENERAL COMMENTS	pg 2- line 11; pg 5 - lines 29 - 32: here and at other places in the MS, you use the term "molecule" when describing absorption of Ca and/or Mg atoms or ions. Also pg 15 - line 13. These all need the same, accurate term. pg 2- line 13: modify should be modifies, I believe. pg 2 - line 25: I think the abstract would be more useful if you gave a number or range of low and high Ca:Mg intake ratios for China and for USA. pg 3 - line 29-30: Perhaps you want to say ". . . among both men and women at a similar Mg intake level but associated with the low Ca/Mg intake ratio in China." pg 5 - line 27: Use CaSR as in line 8 above, or change line 8 to CASR. Also pg 14 - line 46. pg 5 - line 37: number of Ca and Mg IONS? ATOMS? or " . . . if the total absorbed Ca and Mg is relatively constant . . . . pg 5 - line 39: I don't really get your equation. I'd like to do more than trust you, which I do. pg 5 - line 44: do you need both words, cancer and carcinogenesis? pg 7 - line 30: sentence is repeated. not necessary. pg 9 - line 22: I think this would be more clear if the paragraph had a heading or leading sentence such as "determination of low, medium and high exposure categories . . ." to guide the reader and make for easy reading. pg 10 - lines 48 - pg 11 - line 6: This pooled analysis needs a ref or noted that data not shown. pg 16 - lines 3 & 8: Ca "treatment": was it supplements? was it dietary therapy with a high Ca diet? See if you can easily and simply make term this more specific. pg 16 - lines 28 - pg 17 - line 13: this is very good. pg 18 - line 51: I would use the phrase, '(no absorption competition from Mg)" if this is what you mean. pg 19 - line 30 - 32: sentence is unclear: do you mean, "This finding indicates that high Ca plus high Mg, not high Ca or Mg alone, was significantly associated with a reduced risk." ? pg 20 - line 11: I would add to this first sentence: "In the current study of a population with a low dietary Ca/Mg ratio, we found . . ." Maybe even quantify the Ca/Mg ratio. pg 22 - line 32: Would it be correct (and warranted) to add: " . . . a Ca/Mg ratio between 1.70 and 2.63 may be required for (high?; rising?) intakes of Ca and Mg to be protective against colorectal cancer." pg 22 - lines 34 - 42: good. Table 1: do you need to report the baseline factors and demographics for the men at the higher cutoffs of Ca and Mg presented in Table 4? Table 1: in the nutrient sections, you do not tell us what is in the parentheses. SD? SEM? something else? Also, so many significant

	figures!!! for the nutrients. The mind boggles. Also, aren't these "mean" daily nutrient intakes adjusted for age and total energy, etc.??? Tables 2, 3, 4 &5: I assume the parentheses after each HR is the 95% CIs for each, but if so I'd like to be able to confirm this in either the Table title or in a footnote. I really like Figure 1. I wish you could some how put into this visual form your results so far for different mortality rates with various Mg intakes and different Ca intakes in the two type of populations. Maybe in a future paper.

REVIEWER	Sabine Rohrmann Division Head - Cancer Epidemiology and Prevention Institute of Social and Preventive Medicine University of Zurich Switzerland
REVIEW RETURNED	07-Dec-2012

THE STUDY	Some comments/questions: - Why did the authors adjust for age although age was already their time scale in the Cox models? - Introduction: p.6, l.34: "The low Ca/Mg ratio range (below median) in the Western population...". Below the median in the Shanghai study? - In Fig. 1, when printed in black and white, it is not possible to tell which study is which. - Why is the participation rate in SWHS much higher than in SMHS' - Ref. 10, 11 on p.8, l.22 are not properly formatted.
RESULTS & CONCLUSIONS	- It is not quite clear why the authors show results for Mg and Ca in two different categorizations (Tables 3 & 4) - what is the interpretation of the different results? Which one is more important? - p-interaction in the Tables - what kind of interactionis tested? - The Discussion is very long and not well structured. Although the authors have used sub-heading to structure the Discussion, but they do not always stick to these headings and discuss other results as well. For example, the sentence starting on p.16, l. 15 (Consistent with these published findings...) the author refer to their results on total mortality in the paragraph on colorectal cancer. - p. 18, l. 39: " ... in the northern latitude where sunlight is very limited for vitamin D synthesis during spring to autumn." This is not correct - sunlight for vit. D synthesis is limited from autumn to spring. - The authors should try to better differentiate results and interpretation of their finding on Ca/Mg ratio in contrast / in addition to the effect modification by this ratio or the results for Ca and Mg when mutually adjusting. The difference does not become very clear in the Discussion

VERSION 1 – AUTHOR RESPONSE

Responses to the reviewer 1's comments: A group of stellar epidemiologists conducted an study in Asian population to test whether Ca/Mg intake ratio modify the associations between intakes of Ca and Mg with all-cause mortality and cause-specific mortality. The topic is interesting and the paper is well written. I have minor constructive comments as follows:

1: The Objectives in the abstract is worded such that the authors tested the hypotheses on mortality due to gastrointestinal tract cancer. But the results mentioned only colorectal cancer and gastric

cancer. It is unclear whether the hypothesis only applies to these two cancer sites. If not, it seems worthwhile mentioning the results of other gastrointestinal tract cancers.
Response: Thanks for the reviewer's important question. We did not present the results for other GI tract cancers because the sample sizes for mortality due to these gastrointestinal tract cancers are sparse and not reliable. The findings were also not statistically significant. We have added this information in the results section on page 11.

2. Given that postmenopausal hormone use may potentially influence CVD or cancer mortality, is information on postmenopausal hormone use available in women? How results look at after adjusting for hormone use in SWHS?
Response: We have additionally adjusted for postmenopausal hormone use, but the associations did not change. This may be because fewer than 5% of postmenopausal women in SWHS used hormone replacement therapy. We have newly added this description on page 9.

3. Tables already contain comprehensive data. But it might be better to show the number of cases for significant associations when the numbers are relative small for the stratified analysis by ratio (i.e., Table 2, colorectal cancer and gastric cancer) in table footnotes. This will provide some information on how robust the results were.
Response: The number of cases are presented in the tables.

Responses to the reviewer 2's comments: This important study expands the focus of the importance of Ca:Mg intake ratio on the disease preventing aspects of nutritional Ca and nutritional Mg to a population with a low Ca:Mg dietary ratio. Until now, such studies have been limited to populations with a high Ca:Mg dietary ratio. The impact is on important diseases (cardiovascular; cancer) that are rising globally as consumption of the modern process food diet expands.

1. pg 2- line 11; pg 5 - lines 29 - 32: here and at other places in the MS, you use the term "molecule" when describing absorption of Ca and/or Mg atoms or ions. Also pg 15 - line 13. These all need the same, accurate term.
Response: Thanks for the very helpful comments. We have changed "molecule" to "ion".

2. pg 2- line 13: modify should be modifies, I believe.
Response: We have changed to "modifies".

3. pg 2 - line 25: I think the abstract would be more useful if you gave a number or range of low and high Ca:Mg intake ratios for China and for USA.
Response: We have added accordingly.

4. pg 3 - line 29-30: Perhaps you want to say ". . . among both men and women at a similar Mg intake level but associated with the low Ca/Mg intake ratio in China."
Response: We have modified as suggested.

5. pg 5 - line 27: Use CaSR as in line 8 above, or change line 8 to CASR. Also pg 14 - line 46.
Response: We have made corrections.

6. pg 5 - line 37: number of Ca and Mg IONS? ATOMS? or " . . . if the total absorbed Ca and Mg is relatively constant . . . .
Response: We have made corrections.

7. pg 5 - line 39: I don't really get your equation. I'd like to do more than trust you, which I do. pg 5 - line 44: do you need both words, cancer and carcinogenesis?

Response: We have modified the equation. We have removed “cancer”.

8. pg 7 - line 30: sentence is repeated. not necessary.
Response: We have removed “The study was approved by all relevant institutional review boards”.

9. pg 9 - line 22: I think this would be more clear if the paragraph had a heading or leading sentence such as "determination of low, medium and high exposure categories . . ." to guide the reader and make for easy reading.
Response: We have added "determination of low, medium and high exposure categories” at the beginning of the paragraph.

10. pg 10 - lines 48 - pg 11 - line 6: This pooled analysis needs a ref or noted that data not shown.
Response: We have added “Data not shown”.

11. pg 16 - lines 3 & 8: Ca "treatment": was it supplements? was it dietary therapy with a high Ca diet? See if you can easily and simply make term this more specific.
pg 16 - lines 28 - pg 17 - line 13: this is very good.
Response: We have changed “treatment” to “supplementation”.

12. pg 18 - line 51: I would use the phrase, '(no absorption competition from Mg)" if this is what you mean.
Response: We have changed to “no absorption competition from Mg".

13. pg 19 - line 30 - 32: sentence is unclear: do you mean, "This finding indicates that high Ca plus high Mg, not high Ca or Mg alone, was significantly associated with a reduced risk." ?
Response: Yes, we have made the correction accordingly.

14. pg 20 - line 11: I would add to this first sentence: "In the current study of a population with a low dietary Ca/Mg ratio, we found . . ." Maybe even quantify the Ca/Mg ratio.
Response: We have added made the changes accordingly.

15. pg 22 - line 32: Would it be correct (and warranted) to add: " . . . a Ca/Mg ratio between 1.70 and 2.63 may be required for (high?; rising?) intakes of Ca and Mg to be protective against colorectal cancer."
pg 22 - lines 34 - 42: good.
Response: We have added “high”.

16. Table 1: do you need to report the baseline factors and demographics for the men at the higher cutoffs of Ca and Mg presented in Table 4?
Table 1: in the nutrient sections, you do not tell us what is in the parentheses. SD? SEM? something else? Also, so many significant figures!!! for the nutrients. The mind boggles. Also, aren't these "mean" daily nutrient intakes adjusted for age and total energy, etc.???
Response: We have added the full name (standard deviation) for SD in the footnote. Yes, these daily nutrient intakes have been adjusted for age and total energy.

17. Tables 2, 3, 4 &5: I assume the parentheses after each HR is the 95% CIs for each, but if so I'd like to be able to confirm this in either the Table title or in a footnote.
I really like Figure 1. I wish you could some how put into this visual form your results so far for different mortality rates with various Mg intakes and different Ca intakes in the two type of populations. Maybe in a future paper.
Good work! Thank you!
Response: We have added “and 95% confidence intervals (95% CIs)” in the title for Tables 2, 3, 4 &

5.

Responses to the reviewer 3's comments:

1.- Why did the authors adjust for age although age was already their time scale in the Cox models?
Response: We apologize that we did not make it clearer. We adjusted for age to control for potential birth cohort effect. We have added this clarification in the method section on page 9.

2. - Introduction: p.6, l.34: "The low Ca/Mg ratio range (below median) in the Western population...". Below the median in the Shanghai study?
- In Fig. 1, when printed in black and white, it is not possible to tell which study is which.
Response: We have added text to the introduction to clarify which median was used (highlighted in yellow). We have changed the color to grey for ratio in Shanghai and white for the US population respectively.

3. - Why is the participation rate in SWHS much higher than in SMHS'
Response: Although we cannot be certain of the difference in participation, one possible explanation is that women are more likely to participate in health research than men. Further, it is also possible that some of the difference may be due to differences in the Chinese social environment during the periods of study recruitment as the SMHS began 6 years after SWHS.

4. - Ref. 10, 11 on p.8, l.22 are not properly formatted.
Response: We have reformatted the references.

5- It is not quite clear why the authors show results for Mg and Ca in two different categorizations (Tables 3 & 4) - what is the interpretation of the different results? Which one is more important?
Response: We apologize that we did not make it clearer in original manuscript. We found the association patterns with Ca and Mg differed in women (SWHS) and men (SMHS). Because the differential associations could potentially be caused by use of different sex-specific cutpoints we chose to evaluate first the associations using common cutpoints (Tables 2 and 3). As noted, men had higher intake levels of Ca and Mg than women. We also wanted to ensure that the associations were not merely due to this difference in intake. Thus, we have added one additional upper cutpoint for Ca and Mg for men in Table 4. This corresponds to the US RDA for men. We found that highest intake of Mg (>RDA for US men) was associated with increased risk of total mortality and mortality due to cancer. We found this finding was similar to that for intake of Mg >RDA for US women. We have added the descriptions on page 12.

6. - p-interaction in the Tables - what kind of interaction is tested?
Response: Multiplicative interactions between continuous Mg or Ca and continuous Ca/Mg ratio were tested. We have added this description in the method section on page 10.

7. - The Discussion is very long and not well structured. Although the authors have used sub-heading to structure the Discussion, but they do not always stick to these headings and discuss other results as well. For example, the sentence starting on p.16, l. 15 (Consistent with these published findings...) the author refer to their results on total mortality in the paragraph on colorectal cancer.
Response: We have moved sentences to the next paragraph and made additional modifications to improve clarity.

8. - p. 18, l. 39: " ... in the northern latitude where sunlight is very limited for vitamin D synthesis during spring to autumn." This is not correct - sunlight for vit. D synthesis is limited from autumn to spring.
Response: We have changed to “from autumn to spring”.

9. - The authors should try to better differentiate results and interpretation of their finding on Ca/Mg ratio in contrast / in addition to the effect modification by this ratio or the results for Ca and Mg when mutually adjusting. The difference does not become very clear in the Discussion
Response: Thank you for the helpful suggestions to make the discussion more organized and clearer. In the first paragraph of discussion ("Statement of principal findings"), we have added a description to make it clearer that some findings are overall associations after mutually adjusting which are distinct from those effect modifications by Ca/Mg ratio.

VERSION 2 – REVIEW

REVIEWER	Xuehong Zhang, MD, ScD Instructor in Medicine Harvard Medical School Associate Epidemiologist Brigham and Women's Hospital USA No conflict of interest.
REVIEW RETURNED	08-Jan-2013

GENERAL COMMENTS	The authors have addressed my comments and revised the manusript.

REVIEWER	Sabine Rohrmann University of Zurich Institute of Social and Preventive Medicine Zurich, Switzerland
REVIEW RETURNED	22-Jan-2013

GENERAL COMMENTS	I have no further comments.