Author's response to reviews

Title: Calcium plus Vitamin D3 Supplementation Facilitated Fat loss in Overweight and Obese College Students with Very-low Calcium Consumption: A Randomized Controlled Trial

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Author's response to reviews: see over
Dear editor,

Thank you for your kind letter regarding “MS: 1986889335772980 - Calcium plus Vitamin D Supplementation Facilitated Fat loss in Female College Students with Very-low Habitual Calcium Intake: A Randomized Controlled Trial” received on Sept. 21, 2012. We have substantially revised our manuscript in accordance with the comments provided by the two reviewers and in line with the additional formatting request.

ANSWERS TO REVIEWERS:

Reviewer #2

Major compulsory revision

1. *The dose of both calcium and vitamin D was low. Trials of less than 400IU of vitamin D have been shown to be ineffective in relation to bone health outcomes.* *Measurement of plasma 25(OH)D would have helped ascertain effectiveness of the intervention and to evaluate the study outcomes in relation to achieved vitamin D status.*

Answer: We are very sorry for our negligence in measuring plasma 25(OH)D, which might have revealed the vitamin D status in subjects before and after intervention. As for a combined administration of calcium and vitamin D, current literature tends toward the attribution of a larger contribution from calcium due to lack of evidence from clinical trials of the effect of vitamin D on adiposity[35]. Besides, most of interventions administered the dose of vitamin D at 400IU or above, which, as you mentioned, is large enough to be effective for bone health outcomes. Therefore the small dose (125IU) of vitamin D used in our study could only have been helpful in increasing calcium absorption. Due to budget limitations and the fact that some of the study subjects are no longer available (some of them have graduated and have left for other cities), we are unable to get access to these data, which is unfortunate. However, in another on-going cross-sectional study, we investigated calcium and vitamin D consumption of adults, aged 18~74, and also measured their 25(OH)D, PTH, etc. In our future intervention studies, we will give more attention to this issue. Thank you very much for your kind advice.

In addition, the initial calcium intake in the majority of Chinese populations was low. The average calcium intake at baseline in the present study was around 400 mg/d, while in most relevant studies carried out in Western populations the average calcium intake was around 600 to 800 mg/d (or even higher in some studies). In Major’s study[35] for example, the dose of calcium supplements was administered as 1200 mg/d, about 1.5 times of the initial calcium intake. Zemel et al.[14] conducted a 12-week intervention in subjects with 400~500 mg/d of initial calcium intake. The treatment group rose to an 1100 mg/d calcium intake, with the additional 600 mg
provided by yogurt. Therefore, we used an additional 600mg/d, 1.5 times of the initial, in the calcium+D group in expectations that the relatively small dose of calcium supplementation would be effective. Besides, since the calcium supplement used in the present study is an over-the-counter supplement, in which the 125 IU of vitamin D₃ and 600 mg of calcium are linked, therefore we are unable to change the dosage of vitamin D₃ and calcium independently. Necessary changes have been made in Line 216-223, Page 10.

2. Was the supplement vitamin D₂ or vitamin D₃?

ANSWER: It was vitamin D₃. The calcium+D group received supplementation of 600 mg calcium carbonate plus 125 IU vitamin D₃ (purchased from the primary agent of Caltrate in Shanghai, China), administered in a dose of one tablet daily taken after breakfast. We have modified it in the whole text.

3. The statistical power of study in relation to the effect size is necessary to evaluate the largely null results.

ANSWER: On the basis of our preliminary experiment and by referring to previous study of Faghih et al.[19], we needed a total of 42 participants to detect a 1.5 kg difference in 12 weeks in weight change between groups with 90% power at the 0.05 level. We increased the sample size to 26 per group to allow for dropouts with 20% attrition. The formula we used is as follows:

Sample size (per group):

\[
n_1 = n_2 = \frac{2\left(u_\alpha + u_\beta\right)^2}{\sigma/\sqrt{2}} + \frac{1}{4}u_\alpha^2 = 2\left(\frac{u_{0.05/2} + u_{0.1}}{1.96/1.37}\right)^2 + \frac{1}{4}u_{0.05/2}^2 = 2\left(\frac{1.96 + 1.28}{1.04}\right)^2 + \frac{1}{4} \times 1.96^2 = 20.4 \approx 21
\]

21 × (1 + 20%) = 26

Necessary changes have been made in Line 146-149, Page 7.

4. There was considerable loss of subjects, but intention to treat analysis was not used. Whilst use of the latter will bias the study towards the null, intention-to-treat analysis is the norm and part of the CONSORT guidelines.

ANSWER: Only three male subjects concluded the study, which made us take for granted that, with the majority of females (40 subjects), they should be excluded from final analysis from a statistical perspective. Actually, we redid the statistical analysis for 43 subjects instead of 40. The inclusion of three male subjects in the final analysis did not affect significance in the results previously reported. In addition, we reanalyzed the data on body composition changes at 4-week intervals on the basis of the ITT analysis (see Table 3), which indicated that all those who completed the trial...
should be included in final analysis. Necessary changes have been made in the revised manuscript, including the title, as well as in the whole text, tables and figures accordingly.

5. The mechanism used to allocate the sequence of treatment to subjects was not provided. Further detail as to the randomization procedure is needed.

ANSWER: We randomly assigned participants, in a 1:1 ratio, to either calcium+D or control group. Stratified randomization was used when allocating male and female subjects, that is, first, 46 females were randomized into 2 groups with a 1:1 allocation; and then, 7 males were randomized similarly — 3 allocated to calcium+D and 4 to the control group. Randomization was computer-generated by using SPSS 13.0 for windows: Main menu \ Data \ Select Cases \ Random Sample of Cases \ Approximately 50% of cases → Randomization completed. The randomization allocation was carried out by a person that had no involvement in the intervention procedure or data interpretation. Necessary changes have been made in Line 75–77, Page 5.

6. There was much speculation as to potential beneficial effects of dietary protein on calcium metabolism. This is somewhat peripheral to the current study as we had no data on calcium absorption in relation to protein intake.

ANSWER: Following the remarks of to Reviewer #1, “In discussion, the paragraph ‘effect of protein on Ca absorption’ is not related to your issue”, we deleted this paragraph, since the effect of protein on Ca absorption was not our focal point and the discussion section needed to be shortened. Changes have been made in Page 11.

Minor points:

1. More detail of the Food Frequency Questionnaire used to assess baseline calcium intake is necessary.

ANSWER: A semiquantitative FFQ[15] was used for each participant during screening with 149 items in 17 food categories, including rice, wheat flour, other cereals, potatoes, pork, poultry, fish, eggs, milk, legumes and its products, fresh vegetables, salted vegetables, fresh fruits, vegetable oil, nuts, salt, soy sauce and liquor. An average portion size for each item was specified, and subjects were asked about the frequency of consuming that unit throughout the previous year. Dietary records were analyzed, using the Nutrition Calculator Software (version 2.3, developed by the Chinese Center for Disease Control and Prevention [CDC]). Only those whose initial calcium intake below 600 mg received further screening procedure. Necessary changes have been made in Line 91–99, Page 5.
2. *The reason why subjects were recruited with the BMI thresholds is unclear.*

**ANSWER:** We apologize that we did not state clearly in the abstract and title of the manuscript that our subjects are all overweight or obese. Due to previously published studies, which indicated that the effect of calcium supplementation seems to be more pronounced with energy restriction in overweight or obese subjects[10], we conducted this trial in subjects who are overweight or obese, defined by a body mass index (BMI) of 24 kg/m$^2$ or more and 28 kg/m$^2$ or more, respectively, according to the Chinese standard[14-16]. The current study recruited obese and overweight subjects with a lower body mass index (BMI) cutoff, which was believed to be required for Chinese subjects[26, 27], because the Chinese population has a lower baseline BMI to begin with and the risk of cardiovascular diseases is doubled at BMI of 23.0 to 24.9, and tripled at BMI of 25.0 to 26.9[28]. Necessary changes have been made in the **title, abstracts and Line 60–62, Page 4.**

3. *Since body weight was a major outcome measurement in light clothing may not be sufficiently precise. Were subjects asked to void their bladder before measurement?*

**ANSWER:** Yes, they were asked to void their bladders before measurements and to wear light clothes. We revised relevant parts in **Line 106–107, Page 6.**

4. *Comment as to the validity of body composition measures using bioelectrical impedance should be given.*

**ANSWER:** One of our limitations is that we did not use dual energy X-ray absorptiometry (DEXA), the gold standard method for assessing body composition. However, bioelectrical impedance analysis (BIA) is also a validated and reliable method to assess body composition[13, 39]. We have added this to our **Limitations (Line 273–276, Page 13).**

We really appreciated your good comments and kind suggestions.

**Reviewer #1**

1. *In abstract name the metabolic and anthropometric variables.*

**ANSWER:** Necessary changes have been made in **Abstracts**, as follows:

Repeated measures of variance were performed to evaluate the differences between groups for changes in body weight, BMI, body composition, waist circumference, and blood pressure, as well as in plasma TG, TC, HDL, LDL, glucose and insulin concentrations.
2. In background, mention the effects of Vit.D on metabolic and anthropometric changes.

ANSWER: We have made substantial changes in the background section by adding the effect and mechanism of Vit.D on metabolic health and adiposity (See Line 28–49, Page 3)

3. In material and method, what do you mean by “open-label randomization”? According to statistical estimation how many subjects you need? As you had more than 20% drop out (from 53 to 40), has it have any effects on the results of the study? It seems that you did not give placebo to the controls. How do you justify the placebo effect?

ANSWER: We are very sorry we didn’t express it correctly. Ours was an open-label trial with no placebo used in the control group, which is the primary limitation of our study, which might somewhat weaken our conclusions. However, in the present study the dietitians, supervising and giving dietary instructions to subjects, were not involved in any other procedures such as randomization allocation or evaluation of outcome measurements. Subjects in the present study made no communications with each other for their participation is confidential and individual. In addition, we avoided reporting any subjective measurements, such as hunger scores, as well, so as to minimize bias that could occur in an open-label trial. Necessary changes have been made in Line 267–273, Page 12–13.

Randomization was computer-generated, using SPSS 13.0 for windows, with a 1:1 allocation. On the basis of our preliminary experiment and by referring to your previous study[19], we decided that we needed a total of 42 participants to detect a 1.5 kg difference in 12 weeks in weight change between groups with 90% power at the 0.05 level. We increased the sample size to 26 per group to allow for dropouts with 20% attrition. However, during recruitment, 53 participants (instead of 52) were eligible for enrollment.

Initially, we excluded three male subjects, who completed the trial, from final analysis, for we took it for granted that since there was a majority of females (40 subjects), the males should be excluded from a statistical perspective. Actually, we redid the statistical analysis for 43 subjects instead of 40. The inclusion of three male subjects in the final analysis did not affect significance in the results previously reported, however, the dropout rate reduced to 18.8%. In addition, we reanalyzed the data on body composition changes at 4-week intervals on the basis of the intention-to-treat (ITT) analysis (see Table 3), which indicated that all those who completed the trial should be included in final analysis. Necessary changes have been made in the revised manuscript, including the title, as well as in the whole text, tables and figures accordingly.
4.  *In results, figure 2 is redundant.*

ANSWER: In the original version, figure 2 indicated changes on body fat mass in both groups at wk 0, wk 4, wk 8 and wk 12, as the data on wk 4 and wk 8 had not been presented in table 2 or elsewhere in the text, and we thought it was a more direct way to indicate difference between groups. However, we deleted it in the revised version and we listed body composition changes of subjects between baseline and wk 4, wk 8, and wk 12 (at 4-week intervals) on the basis of the intention-to-treat (ITT) analysis.

5.  *In discussion, the third reason seems illogical and the rest of the paragraph is not related to your issue (effect of protein on Ca absorption). So it is better to delete this part. Although there are some articles which show Ca and Vit.D can influence blood pressure, lipid profile and insulin resistance, it is not discuss in this article. Regarding the effect of Vit.D on weight loss and..... whole discussion needs revision.*

ANSWER: We have deleted that part (Page 11), and have made substantial changes in the discussion section. As for Vit.D, current literature tends toward the attribution of a larger contribution from calcium due to lack of evidence from clinical trials regarding the effect of vitamin D on adiposity[35]. Besides, the dose (125IU) of vitamin D used in our study is relatively small, compared to most of interventions which administered the dose at 400IU or above. Therefore, we focused on the effect of calcium in the text.

Perhaps in future, we will future our study for investigating the effect of calcium and vitamin D individually. Thank you very much for your kind advice.

6.  *The manuscript needs to be edited by a native speaker.*

ANSWER: This manuscript has been edited by David Talbert from Canada, a native speaker of English and college teacher.

**Additional formatting request:**

1. Please include a Conclusions section as the last section of the text. This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

2. Please include an Authors Contributions section at the end of the manuscript, before the reference list. We suggest the following kind of format (please use initials to refer to each author's contribution):
3. Please include a figure title and legend section after the reference list.

4. Please include a competing interests section at the end of the manuscript, before the reference list. If the authors have no competing interests, please state: "The authors declare that they have no competing interests."

ANSWER: We have made changes accordingly. Special thanks to your kind advice. All the lines and pages indicated above are in the revised manuscript.

We really appreciate your advice and your time and are looking forward to a reply soon.

Best wishes,

Wei Zhu (First author)