Author's response to reviews

Title: Dietary milk fat globule membrane supplementation combined with regular exercise improves skeletal muscle strength in healthy adults: a randomized double-blind, placebo-controlled, crossover trial

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Author's response to reviews: see over
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Hiromichi Kumagai  
Deputy Editor  
Nutrition Journal  

Dear Dr. Kumagai:

Thank you for your e-mail dated July 18, 2015 regarding our manuscript (MS: 1196492366158306 - Dietary milk fat globule membrane supplementation combined with regular exercise improves skeletal muscle strength in healthy adults: a randomized double-blind, placebo-controlled, crossover trial) together with the comments of the reviewers.

We thank you and the reviewers for the helpful feedback and recommendations, which were invaluable for improving our manuscript. We are delighted to know that reviewer #2 accepted our revised manuscript. We have carefully considered the comments of reviewers #1 and #3 and further revised the manuscript accordingly. We hope that this revision will address the concerns of all the reviewers and that our manuscript will now be acceptable for publication in Nutrition Journal.

Please let me know if I can provide you with any other information. On behalf of all the authors, we thank you very much for your kind consideration and look forward to your response.

Sincerely,

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AUTHORS’ RESPONSES TO THE REVIEWERS’ COMMENTS:

The revised text is highlighted in red font in the manuscript.

Reviewer 1:

We sincerely thank the reviewer for the insightful comments, which we feel have helped us substantially improve the paper.

Reviewer's report

1. I think the authors have made a reasonable attempt to incorporate the reviewers’ comments. However, the expression of the t test seems clumsy and isn't the convention I am used to seeing, does this fi with the journals requirements. The very last sentence lacks sophistication and the thoughts should be more carefully worked into the document. I think the introduction is improved.

Response: We acknowledge the reviewer’s comments concerning the statistical analysis. Following the reviewer’s advice, we re-analyzed our entire dataset using the linear mixed effects model. This analysis revealed a significant difference in leg extension strength and EMG-RMS compared to baseline. There was no significant difference in the interaction of group and period of leg extension strength or RMS.

In response to the reviewer’s suggestion, we have changed our statistical methods from “repeated-measures ANOVA” to “linear mixed effects model”, and we deleted the last sentence considering the results. In addition, we revised the Results (see Tables) and the Methods sections as follows:

(Methods, page 9, line 156)

The differences in study endpoints or the changes from baseline between interventions were analyzed by using a linear mixed model. We included the fixed effects of group, period, period baseline, and the interaction of group and period. We also included subject as a random effect. When the model showed a significant intervention effect, pairwise comparison between interventions was performed with Bonferroni analysis, and changes were considered significant at P < 0.05. For statistical analysis, SPSS for Windows, release 23.0 (SPSS, Chicago, IL) was used.
Reviewer 3:

We are grateful to the reviewer for the thoughtful comments, and have incorporated the suggestions offered into the paper.

Major Compulsory Revisions:

1. The Methods section should include the sample size/power evaluations that were performed to support the chosen sample size of 14 subjects, at least for the primary outcome. This should include the assumptions (e.g. expected treatment effect, standard deviation of the outcome variable, etc.) used and the corresponding power that can be achieved with 14 subjects.

Response: We agree with the reviewer’s comments and regret that we did not describe the sample size information in the original manuscript. Therefore, we have now added information about sample size and a reference (Ota et al.). The Methods section has been revised as follows:

(Methods, pages 9, line 152)

The sample size of this crossover study was based on our previous study investigating leg extension strength after exercise intervention, where the goal was to detect a 5% change. As we used two-sided tests to achieve type I error rates of less than 5%, we determined that 14 subjects would be sufficient to provide a statistical power of 80% in these types of studies.

2. The estimation of treatment effects in the presence of carry-over effects is problematic. While this study does include a washout period (of comparable duration to the treatment period), a significant difference in the baseline muscle strength between the two periods is still reported by the authors, suggesting the presence of such a carry-over effect. It’s also reported that the baseline muscle strength didn’t differ significantly between the treatment groups. The authors should clarify whether this second test for baseline muscle strength between treatment groups was done for each period (particularly period 2). This is of importance as the absence of such a difference at period 2 baseline could indicate the carry-over effect was non-differential.

Response: We very much appreciate the reviewer’s concern regarding carryover effects. In the original version of our manuscript, we had reported a significant difference between the baselines in periods 1 and 2 in our 14 subjects. In the revised
manuscript, we re-analyzed our entire dataset using the linear mixed effects model (see point #4 below). This new method of analysis revealed no significant carryover effect. Therefore, we have revised the Results section as follows:

(Results, pages 10, line 180)

Although the baseline of period 2 was higher than that of period 1 in this crossover study, this apparent carryover effect after the washout period was not significant.

3. As a continuation of the above comment, the authors should include the observed carry-over effect and the potentially insufficient washout period as a limitation in the discussion section.

Response: We agree with the reviewer’s comments. Hence, we included this limitation in the Discussion section as follows:

(Discussion, page 12, line 232)

Second, the washout period might be insufficient because the baseline value of the leg extension strength for period 2 was still higher than the baseline value for period 1.

4. The statistical methods (repeated-measures ANOVA and paired t-test of change from baseline) used in the study are not among the preferred methods in the current literature for cross-over designs where baseline data is available. One preferred option is to fit a linear mixed effects model (e.g. Proc Mixed in SAS) with the dependent variable of either the endpoint (e.g. muscle strength) after 4 weeks or its change from period baseline, the fixed effects of treatment, period, period baseline and the average of period baselines as patient baseline, and the random patient effect. An alternative model that can be chosen a priori when there are concerns of insufficient washout is one similar to the above but with the period baseline and patient baseline fixed effects replaced by the study baseline (i.e. baseline of period 1). In the case of this study, since a carry-over effect is also observed, such a priori decisions between the above two models are no longer possible. Instead, the authors should consider applying one as the primary analysis and the other as a sensitivity analysis so that consistency of results can be assessed.

Response: We agree with the reviewer’s comments concerning statistical analysis.
Following the reviewer’s advice, we re-analyzed our entire dataset using the linear mixed effects model. The new analysis revealed a significant difference in leg extension strength and EMG-RMS compared to baseline. However, there was no significant difference in the interaction of group and period of leg extension strength or RMS.

In light of these data, we changed the statistical methods from “repeated-measures ANOVA” to “linear mixed effects model”, and we revised the Results (i.e., the Tables) and the Methods sections as follows:

(Methods, page 9, line 156)

The differences in study endpoints or the changes from baseline between interventions were analyzed by using a linear mixed model. We included the fixed effects of group, period, period baseline, and the interaction of group and period. We also included subject as a random effect. When the model showed a significant intervention effect, pairwise comparison between interventions was performed with Bonferroni analysis, and changes were considered significant at P < 0.05. For statistical analysis, SPSS for Windows, release 23.0 (SPSS, Chicago, IL) was used.

Minor Essential Revisions:
1. For transparency purposes, is this randomized trial disclosed on public databases such as ClinicalTrials.gov? If so, the registration number of the study should be included in the manuscript.

   Response: We appreciate the reviewer’s question. Unfortunately, however, we have not registered the trial on any public databases at this time.

2. The age range of the study subjects as reported is 31·48 (no mean is provide, see next comment). Although age groups can be defined differently, individuals in this age range are generally considered middle-aged adults rather than young adults.

   Response: We agree with the reviewer’s suggestion. We have therefore changed “young” to “middle-aged” in the revised manuscript.

3. It is good practice in reporting randomized studies to include a table of baseline subject demographics and characteristics (e.g. age, height, smoking status, etc.), as
it allows the readers to better assess the randomization and the population under study. The authors should consider adding such a table by the two treatment sequence groups and for the overall sample.

Response: We greatly appreciate the reviewer’s suggestion; we agree that this would be a useful addition to the paper. We have added the subjects’ demographic information in a new Table 1 in the revised manuscript.