Author’s response to reviews

Title: Novel essential amino acid supplements enriched with L-leucine facilitate increased protein and energy intakes in older women: a randomised controlled trial

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Author’s response to reviews:

Dear Editor,

Thank you for considering our manuscript for publication and for giving us the opportunity to raise the quality of the paper and perfect all sections. We have welcomed all comments made by the reviewers, which we found extremely useful and constructive. All comments have been taken on board and the required changes have been made.

We are providing below a comprehensive response by addressing individually every single point raised while the resubmitted manuscript displays the amended sections/lines in red.

We trust that the resubmitted manuscript has now met all the required criteria needed for reconsideration and communicated through your initial response.

On behalf of all authors

Dr Theocharis Ispoglou

Many thanks for the kind and fair comments of both reviewers, this is appreciated greatly!

REVIEWER 1
As reviewed by the authors, a large portion of older individuals (particularly women) do not meet dietary protein recommendations which may impact frailty rates and contribute to sarcopenia. There is emerging evidence that the EAA leucine in particular is crucial to the regulation of muscle protein synthesis. This report examined the acute effect of a leucine-rich essential amino acid supplement on appetite perceptions and ad libitum food intake in older women. There is little research currently available regarding the palatability of such a supplement and whether this type of supplement impacts appetite and concurrent mealtime energy intake. This is interesting research and the trials were carefully planned and included appropriate outcome measures. Some clarifications are necessary as outlined below.

Comments to be addressed and RESPONSE

1. The supplement ingredients need to be clearly stated. What are the sources of the macronutrients for each of the supplements? Adding a table clearly depicting the ingredients of the supplements would be helpful. Were the supplements purchased from a commercial vendor? The source of the supplements must be provided. Does the micronutrient content of the supplements differ?

RESPONSE to 1: The gel and the bars used are not commercially available. They were developed in collaboration between our university and a product developer to the food industry. Additional details have been included within the main body of the manuscript, whilst two pdf files were submitted as supplementary material. The supplements were provided by Fagron and a main retail provider in the UK (Sainsbury’s).

Yes, the micronutrient content differs due to the different nutrient composition, however for the purpose of this research no micronutrient information is provided. This can be easily calculated from the available nutrient information. To address your comment, the following text has been added within the methods section:

“There were purchased from Fagron UK Ltd, and the remaining materials from a main supermarket chain in UK (J Sainsbury plc). The finished products were developed in collaboration between our institute and a Product Developer to the Food Industry based at Askham Bryan College (UK)”

2. In the Abstract, the supplements should be more clearly described. As stated, the Bar seems not to be rich in EAAs - only the Gel.

RESPONSE to 2: This has been addressed: “both” was added before the statement “rich in EAAs”

3. Verbal confirmation of the diet, fluid intake, and exercise were obtained from participants on the test days (page 5 line 38). These compliance rates should be reported in the results section.
RESPONSE to 3: Further clarification has been provided at the end of the results section to confirm full compliance.

In cases, where participants did not replicate the 24-h diet and exercise followed before the first experimental, testing was rearranged in subsequent experimental trials. Therefore, a full compliance is noted.

4. According to the Control treatment, the amount of the test meal (porridge oats) consumed for breakfast seems low - these individuals were fasting - a more reasonable intake for breakfast seems to be twice this amount. Can the authors comment on the palatability of the porridge?

RESPONSE to 4: As outlined within the results section the different conditions were equally palatable. The closer the score to 100, the worse the palatability score.

The mean range in palatability scores in both experiments were 38-48, 41-47, and 44-46 for the control (porridge), bar, and gel respectively. If you wish, we could report mean values for each condition in each experiment however we believe this will not add any value to the paper. In addition, it is worth noting that there was no difference in the palatability of the breakfast between trials as shown on lines 230 and 274.

Therefore, to address your comment, we have made the following amendments towards the end of the discussion section:

“In line with experiment one the supplements were received well by all participants. The palatability of the breakfast was considered “good” on scoring, and not affected by concurrent consumption of the supplements during the meal”.

In addition, at the start of the discussion, the original statement “The current composition did not negatively affect palatability, smell, taste and aftertaste of the breakfast when consumed alongside porridge meal” was modified to: “The current composition did not negatively affect palatability, smell, taste and aftertaste when consumed alongside a typical porridge-based breakfast meal” to reflect that the porridge based breakfast is a typical meal.

5. For the statistical analyses, were data normally distributed to permit parametric testing?

RESPONSE to 5: Thanks for bringing this to our attention. Yes, data was normally distributed. The following statement has been added in the statistical analysis section to provide clarity:

“Normality was assessed by the Shapiro-Wilk test.”

6. Please cite the 'previous research' that was used to determine sample size (page 10 line 9). What was the SD used for these calculations?
RESPONSE to 6: The following statement has been added in the statistical analysis section:

“…and based on the standard deviation for a similar ad libitum meal to that used within the present study”

The relevant references were also included in the statistical analysis (47 and 49) and the references sections. Please see below:


7. In the Discussion (page 14 line 7), the statement 'instead of protein' seems inaccurate. The Bar contained protein in addition to the EAA, correct?

RESPONSE to 7: Yes, that is correct. Thanks for bringing to our attention. We have changed “contained” with “were enriched”

8. The authors need to discuss the study limitations and delimitations. For example, the sample size is small, and the women were normal weight, free living and healthy. It is not known whether EAA supplementation with a different breakfast menu, or at the lunch or dinner meals, would have the same results. Older men may respond differently.

RESPONSE to 8: Many thanks for your excellent suggestions regarding limitations/delimitations. We would mainly consider them as delimitations rather than limitations, since of most of our decisions were purposeful regarding the inclusion/exclusion of certain individuals/populations. As outlined in the introduction section the main emphasis was to focus at this stage on women since they tend to have larger protein deficiencies than men, whilst women with lower protein intakes are more likely to suffer from frailty. We have also tested a smaller number of men (unpublished data from our lab), and the responses are similar to those of women.

You are absolutely right that it is not known whether EAA supplementation with a different breakfast menu, or at the lunch or dinner meals, would have the same results. However, we had to investigate first what happens acutely using a sensitive standardised meal before we move to the next stage (i.e. how is daily energy intake being affected when the supplements are taken alongside other meals)-for your information, we have just completed another research trial investigating this, and our findings confirm the findings of the current study. As requested, we
have also added the following in the discussion section to shed some further light on limitations/delimitations:

“Despite an adequate sample size for the current study, there is a need for further research to confirm the generalisability and reproducibility of our findings in larger clinical and non-clinical populations, as well as in men. Using a validated sensitive breakfast meal, our findings confirm that both supplements can facilitate an acute increase in protein and energy intake. However, it is still unknown how daily intakes may be affected when the supplements are taken alongside other main meals of varied composition”

REVIEWER 2

This paper suggests supplementation of L-leucine in the form of bar or gel for older women with the aim of improving protein and energy intake, promoting health improvement, immunity and avoiding muscle degradation. In addition, they evaluated appetite regulating hormones, acylated ghrelin, pancreatic polypeptide (PP), and peptidic tyrosine (PYY) to understand the possible mechanism of action.

Although I find it difficult to interpret some figures that are inverted or their symbols are superimposed, I see an adequate and well described structure of the proposed objective. This paper demonstrates clarity in both the discussion and the conclusion, explaining the results adequately and coherently. In addition, the results present promising data for studies related to improvements in the well-being of older women.

Comments to be addressed and RESPONSE

1. Figure 1 - Figure is in black and white, where are the red arrows?

RESPONSE to 1: Apologies for the oversight. We had originally used red arrows that we subsequently replaced with a syringe picture. This has now been addressed.

2. Figure 2 - Legend probably refers to Fig. 3

RESPONSE to 2: Apologies for this. The order of the figures within the main body as well as the numbering of the figures was correct. However, the numbering of the legends was not appropriate. This has now been addressed.

3. Figure 3 - Legend probably refers to Fig. 4

RESPONSE to 3: This has been addressed
4. Figure 4 - Legend probably refers to Fig. 2

RESPONSE to 4: This has been addressed.

5. Are the data of Fig. 3 statistically different? It would be interesting to ask if there is statistical difference in any of the times analyzed.

RESPONSE to 5: Thank you for making this very important observation. We made the decision to analyse AUC values rather than individual time points in accordance with statistical guidance for the analysis of serial measurements (Matthews, J.N., Altman, D.G., Campbell, M.J., and Royston, P. 1990. Analysis of serial measurements in medical research. BMJ, 300: 230–235. doi:10.1136/bmj.300.6719.230. PMID:2106931). Subsequently, individual time points were not analysed, meaning that these findings cannot be displayed within the figure. To address however your point partly, we have added the Holm-Bonferroni adjustment (stated in the stats section of the methods) outputs within the relevant results section:

“Post-hoc analysis, demonstrated that

appetite ratings were significantly higher in CON vs BAR (P=0.012) and GEL vs BAR (P=0.09).”

6. If possible, avoid overlapping the symbols in the figures for better understanding.

RESPONSE to 6: Apologies, but we are unsure where symbols overlap/superimpose. This is not the case with the figures we submitted. However, we would prefer to keep the figures as they currently stand.