Author's response to reviews

Title: Post-Exercise Protein Trial: Interactions between Diet and Exercise (PEPTIDE): study protocol for randomised controlled trial

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Author's response to reviews: see over
We would like to express our gratitude to the reviewers for their valuable time and comments on the current manuscript. Below are point-by-point responses with associated amendments to the manuscript that are highlighted in yellow font and underlined.

**Reviewer 1:**
The study protocol is well described, taking into account the study rationales, objectives and methods.
I have only few minor comments and suggestions in regards to:
1. Experimental design.
   a. I would recommend the author to present the exact information /content/ on nutritional supplements composition used in study phase I and II in the table, similarly to your previous publication[reference 47].

   The nutritional information for the supplements in the current project has been tabulated and added to the manuscript (Table 1; lines 294-296)

   b. Please clarify or supplement the manuscript, whether the mix of nutritional after exercise used in Phase II, also complements the electrolytes or micronutrients?

   The information pertaining to electrolyte/micronutrient content has been added to Table 1 in the revised manuscript. The electrolyte content was not matched between treatments. In contrast to acute-based studies, any differences in electrolyte content between the supplements is not expected to have an influence in a longer term and free living conditions. Particularly when considering the broader view of their daily nutrient intake, which will be examined through the 3x 3-day diet records during the intervention and subsequently compared between groups.

The first morning urine sample or urine sample before exercises will be collected during both baseline and follow-up period?

   The first void urine sample will be collected during the baseline and follow-up measurement days. This is now indicated in lines of the current manuscript (line 425).
3. Muscle biopsy sampling.
I guess the biopsy will be performed under local anesthesia – please specify or cite your earlier publication [47], which accurately describes the technique of muscle biopsy.

We appreciate the comment provided by the reviewer. This was addressed in the current manuscript (lines 484-485 of the revised manuscript).

4. Standardisation of lifestyle.
Please provide more details on dietary and exercise activity record and analysis during the 48 hours preceding the baseline and follow-up tests.
The 48 hours or 3-day records will be assessed?

More detail describing dietary and exercise records have been added to the revised manuscript (lines 509-516).

Both 48-hour and 3-day records are planned to be analysed. This has been clarified in the same section mentioned above (lines 509-516 and line 520).

Discretionary revision
Study objectives and Approach to research question
Describing the two study objectives, please refer rather to the phase of the study (I and II) instead of the numbering format of the style: i, ii.

This has now been updated in the current manuscript (line 141 and 145).

Reviewer 2
Major Essential Revisions

1. Reference 11 reports very similar work to what is proposed here. Please clarify how the proposed trial will extend current knowledge base.

With many thanks to the reviewer, we have attempted to elucidate how our current work will extend current knowledge throughout the manuscript relative to the study by Ferguson–Stegall et al. 2011. Our current project uses running based endurance training (6 weeks) to investigate the effects of post-exercise protein ingestion on endurance training adaptations, a question that remains to be examined in the current literature. Furthermore, Ferguson-Stegall et al. 2011 suggested that the greater
magnitude of endurance training adaptations may have been a consequence of plasma albumin content and plasma volume expansion, albeit this was not directly measured in their study. In our current investigation we aim to explore the changes in plasma albumin and plasma volume in response to the different nutritional interventions. Finally, the precise nutritional interventions are considerably different between our current experiment and that of reference 11 as alluded to in the penultimate paragraph in [background] section.

2. Please provide details on recruitment process and screening for eligibility.
   Further details on the recruitment process and screening for eligibility have been added (lines line 230-240)

3. Please provide detailed eligibility criteria.
   Detailed eligibility criteria have now been included in the current manuscript (lines 250-267)

4. There are only two measurement periods. Thus, ANCOVA, with baseline as a covariate, is recommended. In particular, within group change is not a focus of RCT. I refer to "Analysing controlled trials with baseline and follow up measurements" by Andrew J Vickers, Douglas G Altman.
   Providing the above reference was much appreciated by the authors. Accordingly, [Data analysis] section has been updated to include the suggested amendments (564-570).

5. Please clarify how target VO2 max will be determined during training.
   The target VO2max will be obtained from the sub-maximal and maximal oxygen uptake data during the first baseline visit by means of linear regression. The exercise checklist given to participants will include the calculated speed at any given intensity throughout the training intervention. This has now been included in the revised manuscript (lines 339-342).

6. How will adverse events be monitored?
   The monitoring of adverse events has been added to the revised manuscript (lines 356-361), which reads [Although several risk factors will be screened before
participants take part in exercise, the prescribed physical exercise may increase the risk of certain adverse events (e.g. myocardial infarction, musculoskeletal injuries, etc.). Nevertheless, the prescribed endurance training will be conducted in a gymnasium under supervision of trainers who are familiar with first aid procedures and the occurrence of any adverse events related to the study will be reported.

7. Provide more details as to how compliance will be determined.
Participants will be given an exercise log to confirm each session’s date/time/duration. These individuals will be given and electronic key card to access the gymnasium which can monitor attendance/date/time. The information from the exercise logs will be cross-examined with the electronic monitoring system to verify each exercise session attendance/date/duration. This has now been included in the revised manuscript (lines 543-546).

8. Please clearly state whether participants of Phase 1 will be eligible or not for Phase 2.
This has been updated in the revised manuscript to clarify that participants in Phase I are not eligible for Phase II (lines 247-248).

9. Please clearly state how (e.g., who) will conduct the randomization.
The randomisation will be performed by (JAB) who is an academic supervisor who is not responsible for trial enrolment, nutritional preparation or provision. The only interaction by JAB with participants will be for obtaining muscle biopsies at baseline and follow-up and thus will be unaware of the assigned coding numbers to participants, rendering him unable to determine trial allocation to any participant. This is now added to the revised manuscript; lines 270-274.

Minor Essential Revisions
1. Please have a distinct and separate section for sample size justification.
A separate section of sample size justification has been included in the revised manuscript (lines 554-562).

2. Please clearly state the primary outcome measure of interest on the front end of Methods section.
Statement of the primary outcomes have been included on the front end of the Methods section (lines 157-161).

3. Under the Methods section, keep all the measurements (baseline, follow-up, anthropometric data, etc) grouped together (with appropriate subheaders) and then detail the intervention.
   As suggested, all the measurements have been grouped together with appropriate subheaders).

Reviewer 3

1. P(age) 2, l(ine) 44. State who the two were that were intended to be blind. Double is not specific enough. Also P 7, l 165. Also P 10, l 254.
   With many thanks to the reviewer, the statement now reads [investigator-participant double-blind manner] across the revised manuscript lines 43, 167-168, 281).

2. P 1, l 58. Since [or] logically includes [and], delete [and/]. Also P 14, l 358. Also P 22, l 535.
   The suggested changes have been made throughout the current manuscript (lines 57, 96, 395, 599).

3. P 1, l 60. Specify the date format and include the date the first patient was randomized since l 539 states that randomization has been started.
   The date format has been changed. Furthermore, the date the first patient was randomized has been added in [trial status] section (line 603).

4. P 3, l 83. Rewrite to make recent more specific: [Cycling-based endurance training studies in 2009 and 2011 have ...].
   As suggested, this paragraph (line 82-85) now reads [Cycling-based endurance training studies in 2009 and 2011 have also proposed the role of protein ingestion in supporting ...].
5. P 4, l 100. What about before ingestion?

The authors are aware of only three studies investigating the role of pre-exercise protein ingestion on muscle protein synthesis or net muscle protein balance during a subsequent exercise bout (Tipton et al. 2001 Med Sci Sports Exerc; Tipton et al. 2007 Am J Physiol Endocrinol Metab; Tipton et al. 2009 Appl Physiol Nutr Metab). However, resistance exercise was employed in the aforementioned studies and therefore was not included in our statement concerning endurance exercise.

6. P 8, l 196. Replace [chosen] by [assigned].

As suggested, [chosen] has been replaced by [assigned] (line 200).


As suggested, these changes have now been updated in the revised manuscript (lines 205, 390, 391, 417, 428, 575).

8. P 8, l 210. What method is used to determine optimality?

In consideration of the reviewer’s comment, this paragraph has been reworded and now reads [enhancing the adaptive response to endurance exercise …] (line 214).

9. P 9, l 234 to 236. Should this not wait until the practitioner responds?

This paragraph now reads [Further health screen and a patient-specific direction for the use of anaesthetic will be completed and sent to a medical practitioner, who will respond to confirm the absence of any contraindications prior to any local anaesthesia administration.] (lines 241-243).

10. P 10, l 247. What is the allocation ratio? Under what conditions will the blind be broken if any? What is JAB’s role in the study?

The allocation ratio is 1:1. The blind will only be broken once data collection is completed during each phase.

JAB is an academic supervisor who is not responsible for trial enrolment, nutritional preparation or provision. The only interaction by JAB with participants will be for
obtaining muscle biopsies at baseline and follow-up and thus will be unaware of the assigned coding numbers to participants, rendering him unable to determine trial allocation to any participant. This is now added to the revised manuscript; lines 270-274.

11. P 11, l 267. How do you ensure compliance? Will it be measured? Compliance will be monitored by providing a checklist to confirm supplement intake and timing of ingestion. This has now been updated in the revised manuscript in the [standardisation of lifestyle] section (lines 532-536).

12. P 11, l 276. These sessions should be recorded along with and co-interventions and contamination. The prescribed sessions will be recorded by participants and adherence will be checked via electronic monitoring system that requires participants to use a key card to enter and leave the gymnasium. Participants will be instructed to maintain their regular training and not to perform any exercise on the day of prescribed training. In free living conditions, it is difficult to determine physical activity during a six weeks training intervention. Thus, while co-interventions and contaminants are not precisely recorded, participants will be instructed to maintain their activity habits throughout this period. This information has been included in the updated manuscript (lines 525-526 and 543-546).

13. P 13, l 313. Will consumed water be recorded? Water consumption during the exercise sessions will not be recorded. The ingestion of fluid (i.e. water) may have profound effects on muscle metabolism during prolonged endurance exercise relative to no fluid ingestion (Hargreaves M 1996 Journal of Applied Physiology), thus supporting the rationale for allowing water consumption during training. Given that water ingestion is allowed and that no nutrients that may affect the metabolic response to exercise are provided during the exercise sessions (e.g. carbohydrates), it is anticipated that the metabolic response to each exercise session would be similar between groups.

14. P 13, l 324. Will the results be recorded?
The date of the scheduled meetings will be recorded. In the case that any participant reports any deviation from the prescribed training intervention (missed exercise sessions or supplement ingestion) these events will be recorded and reported.

15. P 14, l 341. Is provision being made for both male and female subjects?
The provision is being made to all participants. This has been added to the revised manuscript (lines 379-380).

16. P 14, l 358. Is there a reference for the method for this bias adjustment? If yes, please include.
There reference where the method for the bias adjustment has been included in the revised manuscript (line 396).

17. P 15, l 385. Is there software that will do this? Cite it.
The linear regression will be calculated on a basic office software (i.e. Microsoft Excel). This has now been included in the revised manuscript (line 421).

This word has been removed in the current manuscript (line 426).

19. P 16, l 409. Delete [systematic] since all bias is systematic
This word has been removed in the current manuscript (line 446).

20. P 18, l 456. What about the 3rd one?
Each muscle sample will be separated into 2 specimens and not 3 (line 490). We apologise for this typographical error.

21. P 19, l 462. Replace [significant] by [important]. This does not appear to be statistically determined.
The word [suggested] has been replaced by [important] (line 501).

22. P 19, l 483. What if they do? Will it be recorded?
The exercise diary provided to participants includes a checklist, which requires them to confirm their adherence to the dietary control that is applied in close proximity to
each exercise session. In the case that this is violated, the number of exercise sessions that were performed under these conditions will be reported once the study is completed.

23. P 20, l 500. Are there any other measures planned? 
Presently, no other measurements have been planned. However, including some other measures (e.g. additional gene expression analysis, etc.) may be considered to further explore any interesting findings.

24. P 20, l 503. What software was used for this sample size? Cite it if used. Why was the stratification by gender not considered?
G*power version 3.1.7 (University Düsseldorf, Düsseldorf, Germany) has been used and is now cited in the revised manuscript (lines 555-556). The stratification by gender was included in the randomisation plan and is now included (line 269) in the current manuscript.

25. P 21, l 521. Suggest inserting [post-] between [by] and [stratification].
As suggested, this section now reads [any meaningful associations (i.e. r ≥ 0.7) between baseline and the magnitude of response to exercise training will be further explored by post-stratification of the treatment group according to baseline status] (line 577).

26. P 21, l 522. Replace [±] by either [,] or [:]. The plus and minus signs are no longer used between a mean and standard deviation as they suggest the reader should be able to add and subtract the standard deviation from the mean to create some numbers that are sensible; when indeed they are not.
As suggested, [±] have been replaced by [;] (line578).

With many thanks to the reviewer for providing this reference, considerations for handling missing data has been included in the updated manuscript (lines 580-588).

28. P 22, l 534. Should [interactions] not be [differences]?
Upon consideration of the second reviewer's comments, data analysis has been updated and thus removing this sentence.

29. P 23, l 559. Insert the grant number if there is one. Also does [Armor Proteins] have any role in the study design, management, analysis or approval of what is submitted for results presentation or publication? Make a statement about this.
   As suggested, the reference number from the funders has been included.
   The nutritional supplier [Armor Proteines] has no role in study design, management or analysis. The supplier will receive any proposed publication or communication citing their material and may provide written comments on the proposed publication, which shall not affect to the scientific value of the proposed publication. This is now added to the acknowledgements section (lines 625-628).

30. A random sample of R(eference)s was chosen to check accuracy of citations.
   P 24, R 8, l 591. Insert [(16)] after [589]. The issue numbers make the Rs easier to find for a reader.
   As suggested, this has now been updated in the references section.

31. P 25, R 11, l 600. Trials likes to cite the first 30 authors before using [et al], so fill in more authors.
   This has been updated.
32. P 25, R 12, l 605. Insert [(11)] after [40].
   This has been updated.

33. P 25, R 13, l 609. Insert [(3)] after [94].
   This has been updated.

34. P 25, R 15, l 616. Insert [(15)] after [586].
   This has been updated.

35. P 25, R 16. What is the current status of this R?
   This has been updated.

This has been updated.

37. P 27, R 34, l 687. Insert [(3)] after [116].
This has been updated.

This has been updated.

39. P 28, R 50, l 739. Insert [(2) after [5].
This has been updated.

40. P 29, R 54, l 749. Rewrite as [Writing Group].
This reference appears to be correct as the name [Evidence Analysis Working Group] is the name reported on the publication (line 819).

41. P 29, R 59 appears to be correct.
Thank you.

42. P 29, R 62, l 771. Add [Correction 2003;4:104.]
Upon consideration of the second reviewer’s comments, data analysis has been updated and thus removing this reference.

43. P 31. Add the stratification by gender after the second box before the randomization
With many thanks to the reviewer, this has been added to the updated figure.