Author's response to reviews

Title: Intramuscular fat in ambulant young adults with bilateral spastic cerebral palsy

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Author's response to reviews: see over
We would like to thank the reviewers for their helpful comments made on our manuscript. We have modified the manuscript in response to their comments. The responses to their specific comments are outlined below. We believe that the reviewers contributions have significantly improved the quality of the manuscript.

**Reviewer 1**

1. Abstract-Background: It is important to better establish the rationale for the study with some brief background. Why is the study of intramuscular fat in CP important?

   The background section of the introduction has now been re-written. A sentence has been added to provide a greater rational. Due to the limited word count for the abstract, it was not possible to elaborate a detailed justification for the research in the abstract.

2. Abstract-Background: There is a word missing in the sentence starting with “The objective of this study...” I think it should say “adults with CP” not “adults with have”.

   ‘CP’ has been added to this sentence.

3. Abstract-Results: How was IntraMF “related to GMFCS”? Assuming you mean there is greater percentage of intramuscular fat among GMFCS IIs and IIIs, compared to Is? Since GMFCS is not a continuous variable, it’s not appropriate to perform pearson correlation with that as an independent variable. If, on the other hand, you found differences with an ANOVA, just state that there was significantly greater IntraMF among GMFCS III compared to IIIs and Is.

   The difference in intramuscular fat by GMFCS level was investigated using one-way ANOVA and post hoc tests. This section has been rewritten to make this clearer without the use of the word ‘related’.

4. Abstract-Discussion: While I completely agree with this, you really can’t make any comment regarding exercise based on this study and these findings. However, you can
mention that this is an important finding that might contribute to cardiometabolic and functional comorbidities in this population.

This section has now been updated to reflect this.

5. Background-General: This is a very nice introduction and rationale.

6. Background-Page 3, Paragraph 2: It would be appropriate here to draw-upon the literature that links sedentary behavior to increased IMAT. The paper by Manini et al. is a good example of the negative implications of chronic inactivity.1

References to this literature have now been included.

7. Methods-Participants. How were typically developing controls chosen? Were they matched on age and gender? Ideally, it would be appropriate to also match on BMI, as this is a known predictors of inter/intramuscular fat; however, this would be difficult as many adults with CP are frail. Please add some commentary on this.

Details of subject recruitment have now been added to the methods section. The participants were a convenience sample and therefore were not matched on BMI. The matching of the subject groups has now been added to the limitations section of the discussion.

8. Methods-Data collection. Since MR data is not the only data collected, you need to provide a description of the methods for anthropometric and GMFCS data collection procedures.

Anthropometric and GMFCS data collection details have now been included into the data collection section

9. Methods-Data Analysis. This should only include statistical analysis in this section. Please move the description of MR data processing to a separate section that includes both MR data collection and processing.
This section has now been restructured

10. Results: Paragraph 2. Please provide the coefficient when discussing results from pearson correlation.

The Pearson’s correlation has now been added.

11. Results: Paragraph 3. Again, not sure how “IntraMF fat was significantly related to GMFCS”. Assuming you didn’t run correlation on this. Also, were you able to actually test for differences? Due to the very small number of subjects in each GMFCS category, I would think this would be difficult. Regardless, and although it’s not necessarily incorrect to say “related,” perhaps the best choice is just to state that “Post-hoc analysis revealed significant differences in intramuscular fat for levels of GMFCS, such that...”

This has been updated to reflect this

12. Results: General. Is it possible to provide MR images to illustrate the differences in fat distribution between two representative subjects (i.e., 1 CP subject and 1 matched TD subject)?

MR images have now been added to the results section.

13. Discussion-General: Overall, this is a very nice, lengthy discussion. It might be possible to reduce the number of redundant statements, if word count is an issue. Also please check to make sure abbreviations are populated (e.g., you abbreviated IMCL twice). As previously mentioned, my suggestion is to also refrain from saying that intramuscular fat is related to GMFCS. Rather, you could say that “these findings suggest that intramuscular fat is associated with degree of mobility impairment.” This is probably due to increased SB and less PA in more affected individuals, but could also be due to greater whole body adiposity relative to lean mass. Interesting about the fat content being higher in muscles with previous surgery. You might briefly (one
sentence) discuss the literature pertaining to muscle injury and fatty degeneration (e.g., Medias et al.2)

The discussion has been shortened and the IMCL abbreviation repetition removed. The term ‘related’ is no longer used when discussing the differences between GMFCS levels. References to muscle injury and fatty degeneration have been added.

14. Discussion: Page 13: The sentence starting with “Further to the variability...” needs to be reworked. As stated it is unclear.

This sentence has now been rewritten.

**Reviewer 2**

This paper confirms some increased intramuscular fat compared to subcutaneous fat. Although the paper reports intramuscular fat, it is not clear how the extramuscular but subfascial fat is segmented using this scan. Based on my understanding I think this most likely segments the extramuscular subfascial fat to the intramuscular segment, if this is the case then the terminology might better be more inclusive like muscle compartment fat level.

Intramuscular fat is segmented by manually drawing regions of interest around the muscle. This way only fat originating from within the muscle is included in the calculation for intramuscular fat. This data processing section the manuscript has been updated to make this clearer and example images of the regions of interest used to calculate intramuscular fat and IMAT given. [give an explanation of Dixon]

Also I feel the implication that it is possible to exercise the muscle and reducing the fat is conjecture which is unlikely, based on the multiple abnormal changes which have been reported in spastic muscle, from the muscle fiber, connective tissue, abnormal neuromotor junctions and reduced motor control.

The reference to exercise implications in the abstract has been removed. The suggestion that exercise studies may have important implications in the discussion has
been modified to reflect the fact that the effect of exercise on intramuscular fat in this patient group is currently unknown.

This study does raise the questions of the physiologic impact this might have, however there are no reports of glucose intolerance or diabetes in children with CP. This does reasonably raise the question if this has just never been investigated or if the intramuscular fat in this population has a different etiology or different implication.