Author’s response to reviews

Title: The relationship between lower limb muscle volume and body mass in ambulant individuals with bilateral cerebral palsy

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Author’s response to reviews:

We would like to thank the reviewers for their comments and very helpful suggestions. We believe that they have greatly improved the quality of the manuscript. Our replies to the individual comments of each reviewer are listed below.

Reviewer reports:

Richard Baker (Reviewer 1): This is a well written paper that reports a solid and important piece of experimental work.

The only detail I think that could be usefully added would be to include BMI in Table 1. If the CP group had a higher BMI then the differences could be attributed to a higher proportion of adipose tissue. Calculating a group BMI from the average height and weight suggests that BMI might be higher in the TD group which I think is useful to know.

Reply: This omission was an oversight. Group mean +/- BMI has now been added to table 1. A comparison of BMI between the groups was performed and reported in the original submission, with a close to significant result (p=0.055).
I'd encourage the authors to think a little more about the significance of their findings. The study is important in confirming that the children with CP have smaller muscles than their typically developing peers. I've got a feeling that the data is telling us more than this but am not sure what.

Reply: This section of the discussion has been expanded, with links taken from sarcopenia and loss of function in typically developing adults.

It looks to me as if none of the regression lines would fit much less well if forced to go through the origin which would suggest that the mass normalised muscle volume is lower in CP than the TD by a fixed percentage independent of weight/age (different for each muscle though). Given that the introduction places the purpose of this study in terms of known deterioration in gross motor function do the results help us understand this any more? If the mass normalised volume is deficient by a fixed percentage regardless of mass/age then presumably it doesn't.

Reply: The origin is within the confidence intervals of the constant for both the CP and TD group, although it is near the upper limit for the TD group. Setting he constant to zero in the model would results in a constant percentage difference in normalised muscle volume between the groups. However there is little evidence to support fixing the constant to zero. Zero body mass is well outside of the range of body mass investigated and we would be predicting that muscle volume and body mass behave in a linear manner throughout early life. Setting the constant to zero may add an artefact to the data that is misleading. A discussion of the intercept and not setting it to zero has been added to the discussion section of the manuscript.

The referee makes a very sound point about the effect of fixed percentage deficit in the group with CP. However, probably due to the non-zero offset in the relationship between body mass and muscle volume, the % deficit of muscle volume relative to body mass increases with body mass. We have given an illustration in the Discussion.

Were other parameters measured (fibre length, physiological cross section)? It would be a shame to have to wait for another publication if they were particularly given that the data presentation is reasonably straightforward and could easily be extended to different parameters?
Reply: Unfortunately these parameters were not measured for these individuals. It is not really possible to measure PCSA with MRI without tractography. We did not use an ultrasound system in this study.

Kristina Tedroff (Reviewer 2):

In this project the authors have investigated the volume of 9 lower limb muscles in 50 individuals with and without CP, 10-23 years old, through measurements obtained by measuring MR scans from the legs. Volumes obtained were then plotted against the body weight of the individuals at one time point. Graphs were constructed that displayed that with increasing body weight individuals with CP had a less steep increase in muscle volume as did the typically developed individuals. This is potentially a clinically important finding and data is presently lacking for these paricular ages. Unfortunately though the authors haven't been able to convincingly show us the possible clinical utility of the findings nor the reliability of the method. Thus prior to this article can be published there are a set of questions and issues that needs to be addressed. Other important covariates and mediators of muscle volume needs to be addressed, these are ethnicity and pubertal development.

Particularly important is the later in this study were in both groups a large majority (~75%) were male, and in the typically developed group individuals were significantly older, heavier and taller.

Reply: Although both groups were predominantly male, which will limit the generalisability of the results of the study to female individuals, the number of males and females within each group were the same (19 male).

We agree that pubertal stage may contribute to the variation in muscle size. However, the strong relationship between muscle volume and bodymass found in this study suggest that puberty is not a major independent factor influencing muscle size in this group.
The volunteers in this study were predominantly white Caucasian. One subject in the CP group was mixed race and one TD subject was black. We agree that there may be differences between different ethnicities as stated by the reviewer, however the volunteers in the groups have similar ethnicity and therefore performing a comparison between the groups is appropriate. However, caution should be used when extrapolating to other ethnic groups. We have add this to the limitations section of the discussion.

The TD group was significantly older, taller, and heavier than the CP group. Individuals with CP are typically shorter and lighter than their age matched peers. Although the mean age was different between the groups, the age range of the volunteers were very similar between the groups. To account for any potential bias due to age, age was included in the statistical analysis and was found to not be a significant factor. What facilitates comparison of the relationships between muscle volume and body mass is the significant overlap in the body mass range of the two groups (Figure 1).

Background

P3, line 15; comma around reference is missing

Line 22; Full stop after 8 years is missing. Word " to" is missing after limited.

Line 24; for consistency compared to line 15 reference should be added after Day et al. OR better still add all references at end of sentences.

Reply: These typing errors have been corrected.

First paragraph two last sentences, I think this section would benefit if you could explain to the reader if and how there is a relationship between muscle volume and strength. If so this would also help supporting why this study is important.

Reply: This has been added to the manuscript.
P5, line 4,5; references missing

Reply: This has been added to the manuscript.

Please state the scientific rationale for the Study. Why did you want to do it? What was the aim?

Reply: This has been added to the manuscript.

Methods

Participants

P6, line 10; how many male?

Reply: 19. The same number in each group. This has been added to the manuscript.

Data collection

Why did the protocol for the MR scanning change twice during the study period?

Image analysis.

Reply: The imaging protocol changed due to availability of MRI scanners and developments to imaging protocols. This is discussed in the limitations section of the manuscript.

P 7, line 27; please change lateralis composite to vastus lateralis. Also be consistent with Table 2 in naming muscles.

Reply: Thank you for bringing our attention to the inconsistent naming of the vastus lateralis and intermedius composite. This has been corrected throughout the manuscript including in table 2.
In the second paragraph of this section the authors describe a method to assess the repeatability of the imaging technique. However, it is not the imaging technique that is being evaluated but the volume assessment method.

Reply: This has been updated to state ‘repeatability of the image analysis technique’.

But furthermore, the method is not clearly stated and thus it is not easy to understand in what way the repeatability or rather reliability (interrater and intrarater) of the volume assessment method has been done. It does seem though as if composite scores and averages were used in a way that possibly can even out differences. A method were a minimum of 10% of the original muscle volume assessments (full assessment 9/research individual) were redone in the same manner by a second (inter rater) or the same (intra rater) assessor would be a better way of ensuing the consistency of the volume assessment method.

Reply: We have modified the repeatability analysis following your advice. We have now reported the inter-rater variability of the results comparing volume measurements performed by the first assessor with the measurements made by a second assessor.

Statistical analysis

P 8, line 8; Please show in detail how MDC was calculated including what data that was utilized.

Reply: We have added equations to the manuscript detailing how MDC and SEM were calculated.

P 8, line 13-16; Second paragraph first sentence- this sentence should be moved to other part of methods. If a subsection named muscle volume analysis or such was created everything pertinent to the muscle volume calculations could be moved to this section. At present this is a bit scattered.

Reply: This has been moved to the image analysis section of the methods as suggested.
In addition this first sentence, line 13-16, seems to state some sort of composite muscle volume score? If this is correct I suggest that you clearly state this and address the score in such a way (it would be beneficial with a name). Is this utilized in figure 1 were it says muscle volume only?

Reply: We have modified the introduction of the single measure to include the statement ‘from here on this will be referred to as lower limb muscle volume’ to make this clearer to the reader.

Results

* I believe the Results section would benefit from some restructuring. For example, the section on muscle volume - the main results of the study- should be moved forward from page 10 to page 9 while the section starting with ICC should be moved back.

Reply: We have restructured the results section as suggested.

* Muscle volumes should be related to BMI rather than weight*length. Please add these data.

Reply: Body mass, and mass*height have been shown to be strong predictors of muscle volume in typically developing individuals by Handsfield et al 2014 (J Biomech. 2014 Feb 7;47(3):631-8. This has also been found in our dataset. BMI is not a strong predictor in muscle volume and therefore we have not presented this data. BMI in our typically developing subjects had an R2 = 0.19 and therefore is not an appropriate variable to investigate muscle growth.

the first paragraph;

Ethnicity should be accounted for in table 1. and discussed in Discussion. Different ethnicities have different muscle mass at the same body weight and length. As an example Afro Americans have higher muscle mass than whites and have different (higher) cut offs for overweight and obesity.
Reply: 24 individuals in each group were white caucasion. One subject in the CP group was of mixed race and one subject in the TD group was black. Ethnicity has been added to table 1 and a discussion added to the limitations section of the manuscript.

BMI should be added to table 1.

Reply: BMI has been added to table 1.

When prior treatment is described please use median and range rather than mean with two decimals.

Reply: Mean has been replaced by median and range as suggested.

Results second paragraph

This entire paragraph is quite difficult to follow. When MDC I stated and average muscle volumes are given it doesn't say if it is TD or CP muscles? Is it all muscles from all individuals averaged together? Can this paragraph be switched for a table with a clear and instructive "legend"?

Reply: These results have been moved to a new table, Table 3. The repeatability analysis was performed by combining the CP and TD muscles to provide a representative range of muscle sizes investigated using the technique.

P 10 and all through manuscript. You might want to switch to a "People first language" writing groups with CP rather than CP groups.

Reply: We have updated the manuscript as suggested.
Discussion

The discussion could benefit from some additional structure. An idea could be to focus more on why you think this is important information and in what way it can help patients and clinicians in decision making.

* Please remove word representative from first sentence since we cannot say with available information that either group are representative of their respective norms.

Reply: The word representative has been removed from this sentence.

* An important mediator to muscle volume in males in particular is puberty. During puberty there will be an increase in lean skeletal muscles and reduction in body fat. This is illustrated by an increase in BMI. Thus a young man has “more muscles” to the weight than a young boy. However puberty occurs at different age and in men it is often not completely finished until 20yrs+1.

* In this study the TD was 3 years older than the group with CP and ca 75% in both groups were male, thus pubertal status can be assumed to be an important factor that cannot automatically be substituted for age (and not age only up to 17). Since this was not investigated it needs to be carefully discussed and evaluated.

Reply: We agree that puberty stage is likely to add some variability to the results of the study. We did not have ethical approval to assess pubertal stage. One option could be to ask the participants to complete a pubertal staging questionnaire, however, these ar unreliable (Desmangles et al 2006 ‘Accuracy of pubertal Tanner staging self-reporting’). We have added pubertal staging to the limitations section of discussion.

* In addition ethnicity needs to be discussed (please see my previous comments from results).

Reply: This has been added to the limitations section of the manuscript.
* GH (growth hormone, an important anabolic hormone) deficiency in CP has been shown in many studies. This could be potentially interesting to discuss as well.

Reply: Growth hormone deficiency is an interesting subject that requires investigation in relation to muscle size in cerebral palsy. We have added a brief discussion of growth hormone to the limitations section of the manuscript.

* It seems like the biggest difference in Muscle volume vs body weight is found in the gastrocnemius muscle. Why is that? Could the individuals with CP have had BoNT-A treatment or surgery to their calves? Is this possible for you to address and discuss?

Reply: Some of the individuals have undergone botulinum toxin injections or surgery to their calf muscles. It is possible that these interventions may explain part of the variability in muscle volume not accounted for by body mass alone. However, the number of subjects in this study is too low to enable the groups to be separated to investigate the effect of intervention. Furthermore, this would also require a control group of individuals who were indicated for intervention but did not receive the intervention.

* It does seem important to be able to evaluate muscle volume in a standardized manner—this is why the method and its reliability needs to be further described and investigated.

Reply: The repeatability of the image analysis technique for calculating muscle volume has been updated following the reviewer’s earlier recommendations.

P13, line 51, Should be GMFCS level III? (says 5)

Reply: Yes this should be III, this error has been corrected.

P14, line 2+3, please rephrase; instead of "…related to the severity of the original injury" write "…related to the severity of the motor disability"
Reply: This sentence has been updated as suggested.

Study limitations
Implications of not assessing puberty needs to be assessed.

Reply: P 14 last sentence. It is likely that puberty may have influenced the results
A discussion of pubertal stage not being assessed has been added to the limitations.

P 15 can a supplementary table with interventions be added?

Reply: A table summarising the interventions has been added to the supplementary materials.

Figure 1.
* Please state on X-axis unit for body mass.
* Is it possible that explanatory description is faulty - symbols mixed up? Curves seems reversed?
* On Y axis is this a composite score of muscle volume?

Reply: These formatting errors have been corrected. Yes this is the composite score for muscle volume. This has been made clearer in the legend.

Figure 2.
* Description of symbols is missing
* X- axis for lateral gastrocnemius differs from other muscles- mistake?

Table 2
* Please use the same names for muscles as in the article text.

Reply: The symbol description is within the legend. The naming convention is now consistent throughout the manuscript.

Table 1

* Add BMI

* Add Ethnicity

Reply: These have now been added to table 1.

Supplementary material

Instead of height mass product I suggest BMI or BMI as well. Please display Muscle volume relationship to BMI graphs.

Reply: BMI is a very poor predictor of muscle volume in typically developing individuals. Therefore BMI is not a good variable to utilise when investigating muscle volume. In our data set, BMI was poorly related to muscle volume with R2 = 0.19.