Author’s response to reviews

Title: Size-adjusted muscle power and muscle metabolism in patients with cystic fibrosis are equal to healthy controls – a case control study

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Author’s response to reviews:

Enclosed please find a point to point response to both reviewers. All comments have been Address and the requested changes and suggestions have been done and marked within the manuscript.

Response to Mathieu Gruet (Reviewer 1):

Abstract:

* first sentence: "reduced muscle function" is weird, i suggest to replace by "skeletal muscle dysfunction” or "peripheral muscle dysfunction"

This has been changed as suggested.
* Wingate anaerobic test to assess muscle "power" is more accurate than "muscle function". Correct also within the manuscript if relevant.

This has been changed as suggested.

* In the results of the abstract: would be great to give some exact p-values when relevant.

We thank the reviewer for this suggestion and added p-values to the results section.

* Conclusion: I still believe that "muscle function" is too vague here. Because for instance, it may also include muscle endurance and fatigability and I would not say that "it is well established ("well-known") that CF patients have alterations in muscle endurance" for instance. So try when applicable to change muscle function by the appropriate terms (eg power, strength, etc…)

This has been changed as suggested.

* P6 "One important confounder in the assessment of muscle function and exercise capacity is the adjustment of the test results for variations in body size" : in body size but also "muscle size" right?

This has been changed as suggested.

Background:

* Reduced aerobic and anaerobic capacity implies exercise intolerance, so there is redundancy here, I would thus rather say, for instance […] "associated with exercise intolerance, including both reduced aerobic and anaerobic capacities", etc…
This has been changed as suggested.

* Hypothesis: "hypothesis was that muscle function and metabolism would not be clinically significantly different between CF and controls"  I guess you mean "would not be clinically significantly different between CF and controls when accounting for likely differences in body/muscle size? Because you said in the revision that you initially expected larger differences in muscle metabolism during exercise between CF and controls, so I guess you expected differences in absolute values but not when appropriately normalized, right? So I suggest to slightly reformulate the hypothesis.

This paragraph has been rephrased as suggested.

* Regarding the use of the wingate test: instead of saying (eg in the discussion) that is a way to have more details regarding muscle function in CF, it would be great to explain why both approaches (local quadriceps test vs wingate test) are complementary?

We thank the reviewer for this comment and added some further explanations to the discussion to further emphasize and clarify our approach.

Methods:

* Sorry if I missed it but could you specify how you chose the increments for the CPET (eg absolute, relative increments?)

We apologize for not having displayed this information earlier: Starting work load was 15 W in patients smaller than 150 cm and 20 W in patients taller than 150 cm; every minute, the load was increased by 15W or 20 W, respectively. This has been added to the methods section.
and what is the version of the wingate test you used? (i.e. duration). The latter is of importance as according to the duration (eg &gt; or &lt; 30-s) all versions of the wingate test may not all strictly rely on the sole anaerobic metabolism, and for the longest versions of the wingate test, dyspnea (in patients with respiratory issues) may be a confounding factor.

All participants performed a Wingate test lasting 30 seconds. This information is now included in the methods section.

Discussion:

* It is a bit weird to begin the discussion with sentences dealing with CPET as it is not the main finding of the study. Especially because you have a lot of results, I suggest to start the discussion with one or two sentences that summarize the main findings of the study.

Main results are now summarized as a short section at the beginning of the discussion as suggested.

* End of the discussion: still not a big fan of the word "sprint group". Could you find something more appropriate here?

We apologize for not having changed this term earlier. It has now been changed to “subgroup performing the high intensity knee extension test”.

Conclusion: I think it would be great for such kind of study which is rather mechanistic to have few sentences regarding the clinical implications of the findings. How the knowledge that peripheral muscle dysfunction is CF is mainly related to a quantitative issue may influence the package of care of these patients?

We thank the reviewer for this important comment. We added a potential perspective to the conclusion section speculating on how these findings may find their way into the care of patients with CF.
Table legends: it depends on the journal guidelines, but consider to list the abbreviations in the legend for each table.

We thank the reviewer for this comment and adjusted the abbreviations section.

Response to Reviewer Jana DeBrandt (Reviewer 2): Dear authors, I would like to thank you for your extensive responses to my queries. My main concerns were addressed. I however have some minor queries that need to be addressed before publication.

In general: Please be consistent in wording about the 'knee extension task'. Sometimes it is not really clear if you are talking about that task.

We thank the reviewer for this comment and changed the wording when necessary.

ABSTRACT

Abstract: line 35: please describe which 'task'

This has been elaborated as requested.

Abstract: line 33: just to make sure I understand correctly: The 15 extra participants for the high intensity task were included on top of the 20 CF / 23 CONT participants? Or you first had 15 CF and 13 CONT and decided to recruit more and add a test? You need to realize that as you didn't power your study and you decided to change your protocol, that your subgroup is in my opinion underpowered. As long as you clearly state this as a limitation in the discussion, I agree for publication. I know that access to MRI scanning is difficult, but at least you should have done a power calculation to know how many patients you exactly needed.
Initially, as you displayed, we had 15 CF and 13 CONT and decided to recruit more patients to add the high intensity task. Due to scarce MRI scanning time slots, we were not able to perform this task with the participants previously included in the study. We are well aware that it would have been great to include more patients and we understand that a power calculation should have been done in advance. Still, we think especially with regard to spectroscopy our data is well able to demonstrate the results found. As requested, we added a further statement on sample size to the limitations section and also included a further statement in the conclusions section.

Abstract: line 47: please include p-value

This has been added

Abstract: line 53: please add ';' instead of ',' before 'power drop' + add p-values

This has been added

Abstract line 60: please describe 'the exercise task in the MRI' as 'the knee-extension task'. Now it is confusing.

This has been changed as requested.

Abstract: page 2 - line 7: please write again 'knee extension exercise or task'

This has been changed as requested.
BACKGROUND

Background: line 10 - page 5: 'constraints in anaerobic and aerobic capacities': please write first 'aerobic' and then 'anaerobic'

This has been changed as requested.

Background: line 15 - page 5: do you mean eligible for 'lung transplantation'? 

To our knowledge, both terms, “transplant” and “transplantation” are equally used for this medical procedure. The term has been changed according to your suggestion.

Background: line 17 - page 5: I would place this sentence 'Interestingly, both, aerobic and anaerobic exercise capacity are related to quality of life [10]' before 'In addition to pulmonary function and physical activity, muscle function is an important predictor of aerobic capacity [9]' as your next sentence is about muscle function and I think the text flows better in that way.

This has been changed as suggested.

Background: line 27 - page 5: What do you exactly want to say with this sentence?: 'Therefore, peripheral muscle function needs to be considered when analysing reduced exercise capacity as studies have proven that improving lung function by bronchodilators does not increase peak aerobic capacity [11] but patients with CF are able to fatigue peripheral muscles despite ventilatory limitations [12]'. I guess that you want to say that reduced exercise capacity in CF is not only because of ventilatory problems but also because of muscle dysfunction? Correct? I struggle a bit with the second part of your sentence when you talk about 'but patients with CF are able to fatigue peripheral muscles despite ventilatory limitations [12]'. What does this mean in regards to exercise capacity if the CF patients are able to fatigue? Please provide clarity.
What we are trying to express is that exercise capacity consists of different components and that peripheral muscle function needs to be considered as one of them. Besides muscle function, lung function is of utmost importance, especially in tasks that have aerobic components. Still, although constraints in lung function are likely to be a major cause of exercise intolerance, peripheral muscle function needs to be kept in mind, especially with the knowledge of the two studies cited: (1) Improving lung function does not improve peripheral muscle function (Dodd, JD et al, J Cys Fibros 2005), and (2) peripheral muscle can still be fatigued despite decreased lung function (Gruet, M et al, Eur J Appl Physiol 2018).

Since, as requested, we changed the two preceding sentences (see response above), the flow is a bit interrupted now. Therefore, we rephrased this section to clarify this aspect.

Background: line 47 - page 5: is this sentence 'In fact, in a mouse model, reduced contractile function of diaphragmatic muscle from CF-mice was observed in the presence of an inflammatory stimulus [17]' related to the CFTR gene? If not I would move this sentence one up.

We all do not know in how far the CFTR gene involves muscle function. In this study, the mice were CFTR knock-out mice, therefore we think it is related to the CFTR gene; it has been postulated in this paper that a lack of CFTR in the muscle may contribute to reduced contractile function. This is why we decided to leave the sentence where it is. If the reviewer feels strongly about this, we can of course move it up.

Background: line 42 - page 5: the CFTR gene is also found in healthy skeletal muscle. Is this ever been measured in CF in the quadriceps muscle? I would guess so. If so could you elaborate a little bit?

We thank the reviewer for this interesting comment: To our knowledge, no paper on CFTR in quadriceps muscle exist, especially no elaboration on muscle function or measurements on the latter. It has been proven that CFTR is expressed in smooth and skeletal muscle, its function, though, is not yet clear, which is why we initially decided to design and perform this study.
Background: line 32: page 6: 'We think that controlling for muscle size by ANCOVA is the most accurate approach to assess muscle function during exercise.' I do not think it is necessary to say that you will control through ANOVA. If you just say that you will control for muscle size this is ok I think.

This has been changed as suggested.

Background: line 7 page 7: Please switch the place of aerobic and anaerobic in the next sentence: 'Combining MRI muscle spectroscopy with anaerobic and aerobic exercise...'

This has been changed as suggested.

METHODS

Methods: line 7 - page 8: please write 'oxygen saturation' instead of 'oxygen saturations'. Can you also add manufacturers of your equipment (ECG, oxygen saturation, wingate bike)?

Manufacturers of ECG and saturation were added as requested. The bike was the same for all tasks and is already explained further in the methods section. For reasons of redundancy, we did not add the same manufacturer twice in the one sentence.

Methods: line 7 - page 9: please write the following sentence also in grams per kg body weight like the sentence before: 'Maximal work load was assumed to be 0.12 kg/kg bodyweight in males and 0.09 kg/kg bodyweight in females.'

This has been adapted as requested.
Methods: line 60 - page 10: you already say that you control for height, CF or not and qCSA in the previous sentence. So please remove this sentence: 'They were controlled for the CF status (CF or CON), qCSA, and height.'

As requested, this sentence was deleted.

Methods: line 3 - page 11: can you please explain again which tests you compare exactly. Meaning which data points were exactly taken for the analysis during the high intensity and incremental protocol? Now it is not clear enough.

We thank the reviewer for this question and elaborated on the methodology in the statistics section.

RESULTS:

Results: line 35 - page 11: now you control exercise capacity by qCSA by dividing it. How did you exactly do this? Is this standardly used? Why don't you just do an ANCOVA? I think it is a bit weird to see the unit vo2peak/qCSA ml/min.

We agree with the reviewer that this calculation seems a bit weird, especially, as it is not standardly used. This parameter (vo2peak/qCSA ml/min) can easily be calculated by dividing the VO2 peak in ml by the measured qCSA. Although this calculation is uncommon it reminds of the much more common report of VO2 in ml/kg bodyweight, which is a standardized way of reporting VO2 peak. Since we think that controlling for muscle size is much more accurate than controlling for body weight we chose to report our data that way. An ANCOVA would for sure be another way to describe this data. However, analysis of CPET results is not the primary outcome of this study, it merely serves to describe the study population, which is why we think this way of reporting this data is not only sufficient for the participants characteristics but further introduces the reader into one of the topics of this study which is the reasonable adjustment of test results.
Results: line 45 - page 11: I requested a comparison of the subgroups to the whole cohorts. Now you include together the CF and Control for subgroup and total group (table 5). This data depicted like this is a bit strange I feel. The only reason why I suggested to compare the subgroup CF vs total cohort CF, and subgroup Cont vs total cohort Cont is just to know if they are not completely different from each other on baseline. There can for example be a bias on the subgroup because they were willing to do an extra test, so maybe they were more motivated or had a better exercise capacity or any other reason. So I don't think you need to make a table for this. I would suggest to compare per group (CF: subgroup vs total group) and (Contr: subgroup vs total group) the parameters and see if there is any differences. And just write one or two sentences on this when you talk about the subgroup analysis. For example: high intensity CF subgroup didn't differ from the total CF group

on '..................'

We thank the reviewer for this comment and agree that table 5 seemed awkward. The comparisons were calculated as requested and two sentences containing the results were added to this section while table 5 was deleted.

Results: in general: explorative: I would like to see the linear regression (ANCOVA) with gender (M/F) included. Or can you two separate ANCOVAs? One for all the women and one for all the men? I now this will lower the sample size. But just in an explorative way? As you can now see in your correlation plots, it is really the men in the top on the right and the women on the bottom on the left. And maybe this correlation on the total sample (women and men) together is mainly caused by that. Because you have two clouds and if you draw a regression equation through it, it is quite logically that it is a good fit. So can you just do a correlation for all the men together and one correlation for all the women together to explore if in both men and women the correlation holds?

We thank the reviewer for this comment. However, we are not quite sure what this comment refers to. Is it all the linear regressions in general? Or does it refer to correlations? These are two completely separate analyses.
As displayed in the methods section, controlling for gender is not possible due to the small sample size. Commonly, as a rule of thumb in a linear model, about 10 subjects per variable are considered to be sufficient (see Harrell, F. et al. Stat Med 1984 doi.org/10.1002/sim.4780030207, or Peduzzi, P. et al. J of Clin Epidem 1996 doi.org/10.1016/S0895-4356(96)00236-3 for example).

We prepared the following explorative analysis for you with gender added to the variables: For peak power, gender as well as status (CF /CON) was not significant for explaining variance, whereas, as in the model included in the manuscript, the two other variables (height/qCSA) were. The same is true for power drop and peak work load of the incremental knee-extension task. Only for mean power, gender also contributed to explaining variance, however, only to a small extent (eta square 0.234), whereas qCSA and height explained most of it (eta square 0.392 and 0.276 respectively). We are aware of the problem of unbalanced gender and understand what you mean by exploratory. We added a statement on gender mismatch, however we do not think that results of this exploratory analysis should be added to the manuscript as they are statistically not really clean due to the small sample size.

Of course, we could also calculate all linear models separately for men and women, again with the problem of the then lower sample size which makes a reduction of parameters necessary. This then, would be a totally different analysis.

With regard to the correlation analyses, gender-separate calculations have been performed as requested. As you suspected, correlations are not exactly the same between men and women (correlation coefficients: qCSA-PeakPower: male: 0.738, female 0.484; qCSA-MeanPower: male 0.785, female 0.613; qCSA-max work load MRI: male: 0.676, female 0.374). However, the number of men (n=27) and women (n=16) are not the same in both groups, which may inflict the results of a separate analysis. This is also the reason why we did not perform the separate analysis right from the beginning. We have expanded the already existing statement on the gender mismatch in the limitations section and in the conclusion to account for this difficulty.

Further, to address this gender mismatch, we already displayed male and female participants separately in the figure. This helps the reader to reflect our results with regard to the cohort.
Results: line 24 - page 12: you write 'reflecting moderate to high correlation coefficients for qCSA and peak power, mean power and Lastmax MRT'. I do not see any correlation coefficients reported in the manuscript. Please add.

The correlation coefficients were added to this section.

Results: line 5 - page 13: can you please explain which data points you exactly compared during the high intensity and incremental protocol? Now it is not clear enough. You can also not write P = 0.000, it needs to be P < 0.001

We elaborated on this in the methods section and also rephrased this sentence to further clarify this aspect. The report of the p-value has been changed as requested.

DISCUSSION:

Discussion: line 40 - page 13: please rephrase to 'a localized muscle function test (i.e. incremental exercise test in the MRI) WAS PERFORMED IN COMBINATION with the investigation of muscle metabolism'

This has been changed to your request.

Discussion: line 53 - page 13: I think it is rather a score 'on' on the wingate test than 'in' the wingate test.

This has been changed to your request.

Discussion: line 12 - page 14: please replace 'was' by 'were'
This has been changed to your request.

Discussion: line 37 - page 16: you use the word 'sprint group'. This is the first time you use it. Please refer to a more used term. I guess the high intensity subgroup?

This has been changed as suggested.

Discussion: line 42 - page 16: I think based on the 5 vs 10 subgroup analysis this conclusion 'Still, since data is controlled for muscle size we think that this influence is but marginal' is too free. I would like to see a conclusion more in the sense of: 'more research is necessary needs to confirm this data' and gender needs to be balanced to trust the results completely.

We thank the reviewer for this remark and rephrased the limitations section to account for the gender mismatch. Further, we added the gender aspect to the conclusions section.

TABLES

Table 1: sometimes you put a range and sometimes not. Please be consistent. Please use () brackets for range and [] for %.

This has been done as requested, ranges have been completed.

Table 1: Please replace TLCOC% by TLCO%.

This has been done as requested.

Table 1: Peak heart rate: please round it up to the decimal. Example: 171.0 \ 171 bpm. Same for O2 saturation.
This has been done as suggested, however, we decided to keep the decimals for the standard deviation.

Table 3: sometimes you put a range and sometimes not. Please be consistent. Please use () brackets for range and [] for %.

Table 3 does not contain any means or ranges nor percentages. We assumed you meant table 2 and changed the respective things in table 2.

Table 3: Do not write $P = 0.001$ but $P < 0.001$ in the table.

This has been done as requested.

Table 4: sometimes you put a range and sometimes not. Please be consistent. Please use () brackets for range and [] for %.

Table 4 did not contain any ranges yet. Since you explicitly requested them, we now added them to this table for further insight into our data.

Table 5: I would delete it. See comment above.

As requested, this table was deleted.