Reviewer’s report

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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Reviewer: Mathieu Gruet

Reviewer's report:

I read with great interest the paper by Ruf et al. dealing with muscle function in people with cystic fibrosis (CF).

There is a current debate among researchers and clinicians in the field of muscle and CF around the question of an intrinsic skeletal muscle dysfunction in CF. This debate takes its origins about twenty years ago but has gained a renewed interest with the discovery few years ago of CFTR expression in the animals and humans skeletal muscles (e.g. Divangahi et al, Plos Genet 2009; Lamhonwah et al. Annals of neurology 2010). Expression of a deficient CFTR in the skeletal muscles of people with CF could lead to several negative scenario leading for instance to ionic homeostasis dysregulation, dysregulation of production/utilization of some metabolites (e.g. PCr), that may finally induce early/exaggerated muscle fatigability and exercise intolerance. In a clinical perspective, if such scenarios are true, it means that it will be harder to fully restore skeletal muscle function of these patients, even if correcting for the usual muscle atrophy observed in these patients.

Regarding the literature, some studies suggest the existence of intrinsic metabolic abnormalities in CF (e.g. Erickson et al. Exp Physiol 2015; de Meer et al. Thorax 1995, Selvadurai AJRCCM 2003), whereas other do not (e.g. Werkman et al. Exp Physiol 2016; Decorte et al. JCF 2017). Such problematic has been discussed elsewhere (J Physiol, crosstalk debate 2017; Gruet et al. JCF 2017).

One important methodological consideration is to control for the potential confounding factors related to muscle deconditioning (lack of physical activity) and the associated atrophy. In other terms, it is important to determine if the skeletal muscle abnormalities in CF are independent of muscle atrophy.
The present study by Ruf et al. brings further elements regarding this topic and suggests the absence of a specific deficit when adjusting between group differences for muscle size, which is smaller in the CF population. More specifically, differences in performance and/or metabolism during a (i) Wingate test, (ii) a quadriceps incremental test with low frequency and long stages and (iii) a high-intensity constant load quadriceps exercise, all disappear when adjusting for muscle size.

These results are in accordance with some previous studies (e.g. Gruet et al. JCF 2016; Decorte et al. JCF 2017) which found no differences in neuromuscular function (e.g. muscle contractility, sarcolemmal excitability) and metabolism (e.g. PCr, ADP, pHi, Pi/PCr) between mild-moderate CF patients and healthy controls matched for physical activity levels. However, it is contradictory to other studies (e.g. Selvadurai AJRCCM 2003).

The main novelty of the study by Ruf et al. is to operate a statistical analysis that allows to directly control for the influence of some potential confounders, with an ANCOVA considering quadriceps cross-sectional area and height of the subjects.

Overall, this is a well-designed study, this paper is well written, the analysis is robust, and the message that cross-sectional area is really important to consider when comparing muscle performance is supported by the results of the three different tests.

However, some methodological considerations must be considered and some complementary analyses may be necessary to fully support the present results and the conclusion of this study. Moreover, I also have some suggestions to further improve the introduction and discussion.

* Introduction:

In the general background, the authors mixed findings regarding reduced aerobic capacity and those regarding reduced anaerobic power. When stating for instance that exercise limitations in CF are related to pulmonary disease and other factors such as inflammation, it is unclear if the authors speak in general or rather speak about anaerobic or aerobic capacity. Moreover, the clinical implications of altered skeletal muscle function is not enough developed here and then it is not clear why it is important to focus on skeletal muscle function in this population.

I suggest to slightly reformulate the first paragraph considering the following elements:
1- CF is associated with poor exercise tolerance which has major clinical consequences (e.g. survival, QoL, etc…).

2 - Exercise intolerance concerns both aerobic and anaerobic function.

2A - Several factors can explain reduced aerobic capacity in this population, including ventilatory mechanics and gas exchanges abnormalities. However, it seems that peripheral muscle function is also important to consider (many possibilities here to justify the importance of skeletal muscle function during aerobic exercise in CF: for instance, bronchodilators, despite improving lung function, failed to improve peak aerobic capacity in people with CF, e.g. Dodd et al. JCF 2005, or, symptoms of muscle effort evaluated by RPE scale during incremental cycling exercise are equivalent in intensity to symptoms of breathlessness, and that whatever the intensity of exercise (Gruet et al. EJAP 2018), confirming that despite their important ventilatory limitations, patients with CF are still able to fatigue their locomotor muscles).

2B - Muscle function is also important for anaerobic performance (e.g. all the papers that demonstrated correlations between fat free mass and performance during Wingate test in CF).

3 - Thus, muscle function is a potential important limiting factor of performance in CF, which justify why we must shed light on its specific role. The natural question that arises is to determine whether it is just a quantitative problem (e.g. reduced muscle mass) or whether it is also a qualitative problem (i.e. directly linked to CFTR expression in skeletal muscles and the resultant metabolic abnormalities).

In this context, it is important to investigate the skeletal muscle function in isolation (reducing the potential confounding role of respiratory factors in alteration of muscle metabolism). It is in my opinion worth mentioning, because it is a limitation of studies which investigate muscle metabolism (for instance using NIRS) during whole body exercise. On the other hands, it should be noted that one limitation of using local contractions is the limited ecological validity of the findings [regarding this issue, did you try to correlate some changes in metabolites during the quadriceps exercise, or simply the performance during the quadriceps test (e.g. maximal workload), with the performance during the Wingate test? One strength of your study is that you have, in addition to local exercise tests, a whole body test that is one of the gold standard to evaluate muscle power. Try to establish a link between alterations in local exercise metabolism with a more functional muscle testing (Wingate) is interesting and, to my knowledge, has not been previously reported. It would permit to reinforce the transfer of your mechanistic results to real-word settings.]
4 - In this context, local exercise using MRI is interesting for several reasons. Previous studies found conflicting results, as illustrated by the recent crosstalk in J Physiol 2017 "Skeletal muscle oxidative capacity is/is not altered in cystic fibrosis patients". One way to clarify this important question is to adjust for variations in body size and muscle mass, which has not been adequately performed in previous studies.

I thus suggest to slightly reformulate the introduction considering those elements.

Then, page 5 L56: the authors stated that "only very few studies have adequately adjusted for differences in body size when muscle function was evaluated. In most studies, either no adjustment was employed at all or a ratio to body weight was calculated. This latter approach has been proven to be inadequate [19]."

I think that this should be better elaborated, especially since the novelty of the present study mostly relies on this methodological aspect. I understand the idea that the scaling was not optimal in previous studies. However, I am not sure that we can state that no adjustment has been employed in previous studies, or just body weight was considered. For instance, in Decorte et al., JCF 2017 subjects performed calf exercise with MRI during incremental exercise using absolute increments, and found that the differences disappeared when normalizing for differences in calf size. They also used an exercise at 50% maximal workload performed for 12 minutes. In Gruet et al. JCF 2016, they performed a quadriceps incremental exercise based on a % of maximal voluntary contraction (MVC). By using such exercises based on relative force level, you can compare your groups because differences in strength (and thus muscle mass) will not directly influence the performance. While I understand that scaling using ANCOVA is important and probably better that using a ratio or an exercise performed at a relative force level (as it is used in most endurance / fatigue studies), I believe this should be better elaborated and justified. You also state that the approach which consists to use a ratio of body weight is not appropriate and cite Tanner JAP 1949. In support from this old reference, I think that more detailed explanations are necessary here, especially because the ratio of body weight is something very popular among clinical exercise physiologists. For instance, VO2peak is always expressed in absolute value (e.g mL/min) but also relative to weight (i.e. mL/min/kg). Thus, further explanations here will be very useful for the readers interested by controlling their outcome for body weight / muscle mass, etc…
* Methods:

- I am not sure to understand how and why you used increments ranging for 0.5 to 1 kg during the first quadriceps test. The increment may vary up to a factor of two, which is not frequent when considering usual ways to increment exercise (it would be similar for instance to have increments during cycling CPET that vary from, 30 to 60 watts). You stated that this is based on patient's anticipated maximal workload. How do you quantify / make this prediction? This is an important issue as it may influence the achievement of the last workload, especially because you used very long stages.

- I am very interested by your protocol and the use of (very) long stages. This is a different approach from what it is usually performed in the literature (most of the time it is 1-3min stages). I think it may have an interest to obtain a steady state for some metabolic parameters? This may be further justified here and maybe discussed as a potential novelty as compared to all previous protocols that used shorter stages. One negative aspect of such very long protocol with absolute increments which are not directly based on maximal force or power, is the likely high variability in time to exhaustion. Whereas such exercise may mainly rely on aerobic metabolism in some subjects, it may be different in other subjects for which the first increments are already very hard to perform. Could you please add some informations regarding individual data for time to exhaustion (in both CF and controls) ?

- You state that your design is interesting because you can compare your CF and controls for exercise performed at a similar level. However, despite mentioning that you recorded metabolites at the end of each 5-min exercise bouts, you only report results at (i) pre exercise, at (ii) maximal load and at (iii) recovery. Why not reporting kinetics of changes at isotime at different time points of your protocol? It ensures optimal comparison for instance, after the completion of a given number of stages, or by normalizing your data in percentage of total time duration (e.g. 25%, 50%, etc…). Without such analyses, you only have a comparison with your last stage at exhaustion, which does not occur at the same time point for all subjects. Moreover, especially in fragile patients and by using very long stages, we can expect an influence of motivational factors that may impact the measurements at exhaustion; That is why it is always interesting to consider other time points early in exercise, to be free of this problem of differences in time to exhaustion and the confounding factor of motivation.
- Regarding the subgroup of patients that perform a second quadriceps test at a higher intensity. It is a very good idea to perform such additional test and I agree with the rationale of the authors for doing this. I also perfectly understand why, from a logistical/feasibility point of view, it was complicated to perform it at the whole group level. However, while you control for potential gender differences for your whole group with an approximate comparable number of female in both groups, you have a strong difference with your subgroup (20% of female in the CF vs 60% in the control group). Previous studies suggest differences in endurance / fatigability etc… between men and females and it is always useful to control for this parameter. In CF, it is also possible that females, for differences reasons, may exhibit greater physiological alterations during exercise than male even after considering lower muscle mass (although it is just an hypothesis, but see for instance the particular results of Selvadurai et al. AJRCCM 2003 which found significant metabolic alterations in CF females compared to CF controls, despite similar muscle mass and greater level of habitual physical activity in CF females). Maybe this issue should be underlined as a potential limitation.

- Why using only an 8-min recovery period between the two quadriceps tests? It is possible that all subjects did not start the second quadriceps test with a full muscle recovery and inter-individuals differences in fitness and ability to recover from an exercise conducted until exhaustion may have slightly influenced the results of the second test.

Regarding all these comments, the discussion should be edited to better reflect these methodological considerations and further underline the novelty of the study compared to previous studies.

* Tables: why using the word "sprint" in Table 4

* Figures: maybe it is just on my computer but the resolution of the figure 1 showing the ergometer is bad. Is it possible to reupload with an improved quality?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes
Does the work include the necessary controls?
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