Author's response to reviews

Title: Relationship between daily physical activity and aerobic fitness in adults with cystic fibrosis

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Author's response to reviews: see over
Cover letter

To:
The Editors of BMC Pulmonary Medicine

Dear Dr. Sanjay Chotirmall,

Please find enclosed the manuscript entitled “Relationship between daily physical activity and aerobic fitness in adults with cystic fibrosis” by authors Savi D, Di Paolo M, Simmonds N, Onorati P, Internullo M, Quattrucci S, Banya W, Laveneziana P, Palange P.

Thank you for the opportunity to submit our manuscript as a new submission. We greatly appreciate the thorough review that took place and the relatively unusual circumstance in which you have allowed us to re-submit our work. We have extensively reviewed the study and taken into account all the comments. We have strengthened the data by including more patients and provided more balanced conclusions. We hope the manuscript is now acceptable to you. A full description of our responses to reviewers comment is also attached.

Editors’ comments:

1. suboptimal clarity with regards the primary research question:

   **Answer**: We rewrote the introduction to describe with more clarity the reasons and the aims of our study. The purpose of this study was twofold:

   The first aim was to examine the relationship between daily physical activity (PA) and aerobic fitness both at submaximal and maximal levels in clinically stable adults with CF. Specifically, we were interested in evaluating the possible utility of PA as a surrogate of CPET in the aerobic assessment of CF. (Background, Page 3 lines 22-25).

   **Rationale**: The best clinical practice to investigate aerobic fitness includes measurements obtained during CPET, however there are still many difficulties associated with its use in clinical practice (Wasserman, Am Rev Respir Dis 1984). Submaximal exercise related data (also known as lactate threshold-LT measures) is an alternative index of aerobic fitness, information that is directly relevant to a patient's ability to carry out the tasks of daily living and may be useful in the clinical environment in patients who may not be able or willing to provide a maximal effort, especially when maximal exercise tolerance is limited by ventilatory factors (Wasserman, Am Rev Respir Dis 1984).
Despite some studies highlighting the use of exercise testing in CF and CF guidelines recommends an annual assessment, CPET remains still an underutilised clinical measure (Gruet M J Cyst Fibros 2010; Stevens J Cyst Fibros 2010; Bell SC J Cyst Fibros 2010 Standards of care and good clinical practice for the physiotherapy management of cystic fibrosis. Association of Chartered Physiotherapists in Cystic Fibrosis 2011). So, to investigate this important aspect, different methods of quantifying exercise capacity in CF are required which are not excessively stressful and are acceptable to patients. It has not been investigated in adults with CF whether objective measurement of physical activity could be added among the CPET surrogates. To date, the relationship between formal CPET parameters at submaximal workloads and daily activity levels in CF has not been well established. So, the possibility that measuring habitual physical activity by a portable accelerometer could be used to assess the CF aerobic state is worthy of further investigation and could bear obvious advantages. (Background page 2 lines 9-26, page 3 lines 1-6)

The second aim of this study was to compare both daily PA levels and responses to CPET of adult CF patients and healthy controls. (Background page 3 lines 25-26, page 4 lines 1-3)

**Rationale:** Few studies have compared daily PA participation in adult CF patients and in healthy subjects. The CF population examined by Troosters et al. (ERJ, 2009) revealed they spent less time in moderately intense PA compared with healthy controls. Conversely, our recent study demonstrated that the time spent in daily PA at different intensities was similar in adults with CF and age-matched healthy controls (Savi et al. Resp Med 2013). Similarly, Rasekaba et al. (J Cystic Fibrosis 2013) showed that moderate or vigorous habitual physical activities measured using a validated questionnaire were similar between CF adults and controls. So, it remains unclear whether CF adults have different habitual activities compared with healthy peers and in order to clarify the discrepancies in the current literature we conducted this study. Also, despite our knowledge about the beneficial effects of a supervised exercise intervention on submaximal and maximal oxygen uptake in CF (Gruber W, Eur Respir J 2011; Gruber W, J Pediatr 2011), there have been no studies examining the effect of habitual daily PA on submaximal workload parameters in CF. (Background page 3 lines 8-21)

In analyzing the possible positive effect of habitual PA in CF, we sought to evaluate the parameters obtained at submaximal level of exercise in CF and in healthy control peers. (Background page 4 lines 1-3)

**2. Appropriateness of your selected control group**

**Answer:** Regarding PA levels of the healthy control group, our population seems to be more sedentary than Troosters’s control group (Troosters et al. ERJ, 2009). Despite similar number of steps (10591 in our study versus 10281 in Troosters’s study), PA at moderate and vigorous intensity were lower (moderate PA 11 min versus 34 min and
vigorous PA 1 min versus 9 min in Troosters’s study). We have now included a new sentence in the discussion section of the manuscript (Discussion, page 11 lines 20-26, page 12 lines 1-13).

We highlighted as a limit of the study that our healthy controls are disease-free but possibly more sedentary, even if they were recruited from staff and colleagues of our hospital and they were physiotherapists, students and doctors. Unfortunately, it was technically difficult to recruit a different control group at the time we conducted the study. It is possible that the reasons for the differences in the PA results may reflect cultural differences, subject motivation to participate in the study or an issue of sampling error. (Discussion, page 13 lines 19-26).

3. issues arise over sample size

Answer: The new sample size for our study is large enough to detect differences between CF patients and controls even at 90% power. A previous study by Troosters et al. (ERJ 2009) showed a difference in peak VO2 % predicted of 41 with a standard deviation of 13.62. We proposed that our effect size could be about half of that obtained by Troosters, therefore for 80% power and 5% significance and similar variability would require a total 36 subjects in a ratio of 2:1, we therefore recruited 45 in total in a ratio of 2:1.

So our new population studied is 30 CF and 15 healthy subjects. We have now included a new sentence in the Methods section of the manuscript (Study Design, page 5 lines 10-15).

4. I also share concerns about the lack of Bonferroni corrections not applied to the statistical analysis and that some of the described results have been over-interpreted.

Answer: We did not apply any specific test like the Bonferroni correction to adjust the p values for our comparisons, however we took a pragmatic decision to set the significance level at $p \leq 0.01$ to correct for multiple comparisons. We have now included a new sentence in the Statistical Analysis section of the manuscript (page 8 lines 1-2).

Reviewer 1:

Reviewer's report
Title: Association of daily physical activity and exercise performance in adults with cystic fibrosis

Version: 1 Date: 31 August 2014

Reviewer: Theodore Dassios

Reviewer's report:
Savi et al conducted a prospective case-report study examining the possible usefulness of measuring habitual physical activity by a portable accelerometer in
assessing aerobic state by comparing it to established measures of cardiopulmonary exercise testing. They also compared responses to maximal and submaximal exercise testing in CF patients and healthy controls. In concept, this is a very interesting study and the idea of possibly substituting maximal exercise testing with submaximal measurement of habitual activity in the assessment of aerobic capacity in CF certainly bears obvious advantages. However a number of concerns on the methodology of the conducted work might put the validity of the authors’ conclusions into question.

MAJOR COMMENTS:
1. Pre-existing aerobic state
   One of the paper's major conclusions is that CF patients and healthy controls have similar aerobic responses to both maximal and submaximal exercise. Although the authors have compared the levels of pre-existing aerobic capacity in the two groups (CF and health) and reported similar levels of activity and aerobic responses, it is not clear what is the starting aerobic state in each group: i.e. it might be that aerobic responses in CF patients are comparable to healthy controls because CF patients exercise and are in a better aerobic state than healthy controls that are disease-free but possibly more sedentary. With regards to this, the authors state in their discussion that CF patients are “encouraged to exercise and have an active lifestyle” (although not within a structured aerobic programme) and in their results they report that CF patients have more time and metabolic equivalents of physical activity compared to the study’s control group. So, one is left with the question: To what degree does the pre-existing aerobic state in the two groups affect the results? Is this a draw between sedentary/healthy on the one hand and active/CF on the other?

Answer: Thank you. CF patients and controls reached their lactic threshold (LT) at a similar V'O\textsubscript{2} and work rate; conversely, they stopped exercise at a lower peak V'O\textsubscript{2}, work rate and HR than healthy controls. So our study demonstrated that, despite reduced exercise tolerance, CF patients presented V'O\textsubscript{2} responses to CPET comparable to healthy controls and reached their LT at a work rate and O\textsubscript{2} uptake similar to that of healthy controls. This is important if we consider that the V'O\textsubscript{2} at which lactate starts to increase closely reflects the fitness of an individual and can increase with a training program (W. Gruber, ERJ 2011). Moreover, despite our knowledge about the beneficial effects of a supervised exercise intervention on submaximal and maximal oxygen uptake in CF [Gruber W, Eur Respir J 2011; Gruber W, J Pediatr 2011], there have been no studies examining the effect of habitual daily PA on submaximal workload parameters in CF.

The novelty of the present study was that we observed these CPET responses in a group of adults with CF who were not engaged in specific home-based exercise training but were generally active as part of their daily lifestyle. This study was conducted recruiting adults CF patients consecutively from the CF outpatient clinic and healthy control subjects from staff and colleagues of the same hospital by invitation. CF patients and healthy controls were asked to continue their habitual daily activities. We underlined that they were not engaged in a specific home exercise training programme. We did not provide any kind of training instructions or materials for the exercise, nor was their
habitual physical activity reinforced by telephone contact. CF patients and controls wore the accelerometer for five typical days doing what they normally do. We have highlighted this concept both in the methods section of the manuscript and in the discussion (Total body activity measurement, page 6 lines 21-23; Discussion, page 12 lines 23-26).

In fact, we evidenced similar levels of daily PA between CF and healthy controls. The sentence has been changed in the revised manuscript (Results, page 8 lines 16-19). We can conclude that the pre-existing aerobic state in the two groups was similar.

To resolve issues arising over sample size, we confirmed these results after studying 30 CF adult patients. We have now included a sentence in the methods section of the manuscript as the reviewer indicates (Study design, page 5 lines 10-15).

Regarding PA levels of the healthy control group, our population seems to be more sedentary than Troosters’s control group. Despite similar number of steps (10591 in our study vs. 10281 in Troosters’s study), PA at moderate and vigorous intensity were lower (moderate PA 11 min vs. 34 min and vigorous PA 1 min vs. 9 min in Troosters’s study). It is possible that the reasons for the differences in the PA results may reflect cultural differences, subject’ motivation to participate in the study or an issue of sampling error. We have now included a new sentence in the discussion section of the manuscript (Discussion, page 11 lines 20-26, page 12 lines 1-13).

We highlighted as a limit of the study that our healthy controls are disease-free but possibly more sedentary, even if they were recruited from staff and colleagues of our hospital and they were physiotherapists, students and doctors. Unfortunately, it was technically difficult to recruit a different control group at the time we conducted the study (Discussion page 13 lines 21-26).

The correlations between CPET parameters (at least for the V'O2 response) at submaximal level of exercise and PA and their similarity with those observed in our healthy controls, made us assume that habitual PA may positively affect the submaximal exercise parameters in adults with CF. We have now included this sentence in the discussion section of the manuscript (Discussion, page 13 lines 10-13).

2. Matching

The authors have used a rather small group of patients (16) and they have compared this with 15 controls that are actually not matched for age and gender with the patient group. In such a small sample, the fact that 25% of the CF participants are female compared to 33% of the control subjects and the fact that mean age differs by 3 years (which might affect disease progression and evolution of aerobic capacity) might have a significant impact on the results when it comes to aerobic or muscular state. For example, one might assert that hand grip force is similar in CF and controls because in the CF group there are more males (higher muscular force in the males but decreased muscular force in CF compared to healthy individuals). Although it might be technically difficult to recruit a different control group at this stage, these points need to be highlighted.
Answer: Thank you. After reviewers’ comments of our paper, we recruited more CF patients (n=30). In our new sample CF males were 20 (67%) and healthy control males were 10 (67%). CF females were 10 (33%) and healthy control females were 5 (33%). So, controls are actually matched for gender with the patient group. Also, there were no statistically differences in term of age between the two groups.

3. Applicability
The authors suggest that aerobic testing in CF could be substituted with PA measurements. (This they do in the introduction in a rather subtle manner and it probably needs to come out more clearly in the manuscript: in the abstract and in the introduction.) Obviously, CPET variables have been reported to predict outcome (authors’ reference 1, 2), but does this hold true for PA as well? Although understandably the authors cannot yet relate their results with future outcome, this is also something that has to be mentioned probably as a limitation of the method at this present time.

Answer: Thank you. As suggested, we modified both the introduction and the abstract and we clearly wrote that we were interested in the evaluation of the possible utility of PA as surrogate of CPET in aerobic assessment in CF (Abstract, page 1 lines 5-6; Background, page 2 lines 17-26, page 3 lines 4-6 and lines 22-25).

We cannot yet relate our results with future outcomes, and we mentioned that in the “limitations of the study” section (Discussion, page 13 line 26 and page 14 lines 1-3).

4. Too many parameters
Are all the parameters mentioned in table 3 needed to support the study’s conclusions? It feels that too many indices are presented and probably the study should stick to fewer indices in a form that could be used as a take-home message. Which one of all these indices do the authors suggest that the clinician should use to substitute CPET with and why? For example: the 6 minute walking test, the hand grip, the breathing pattern, the vital capacity and the HR: Are they all needed to support the conclusions of the study? If not they could be removed for simplicity and clarity.

Answer: Thank you. We have removed hand grip, breathing pattern, vital capacity from the table as suggested by the reviewer.

We compare parameters obtained at submaximal level of exercise (= obtained at the lactic threshold during CPET) between CF and healthy controls. We showed for the first time that CF patients and controls reached their LT at a similar VO2 and work rate. Also, HR and HRR describe if the subjects failed or not to maximally stress the cardiovascular system at the time they stopped exercising. So, all the indices in the new table 2 are important to support the conclusions of the study.

5. Associations
It would be interesting to explore the association of PA with FEV1 and BMI, in order to demonstrate the relation of physical activity with declining spirometry and nutrition (if such a relation is there to report).

**Answer:** Yes, done. Lung function was not related to the PA outcomes or to the CPET parameters at LT. There was a relationship between FEV1 expressed as absolute value and both \(VO_2\text{peak}\) and Watt.

We have now included this sentence in the results section of the manuscript as the reviewer indicates (Results, page 9 lines 7-9).

**MINOR COMMENTS:**

**Abstract:**

1. 6MWD is not explained

**Answer:** Thank you. 6MWT was removed as suggested by reviewers.

2. It should be stated that LT represents “submaximal” level of activity

**Answer:** Thank you. We have specified in the abstract that LT measures represent submaximal exercise related data (Abstract, page 1 lines 12-13).

3. It should be stated that the Sensowear Pro3 is an “accelerometer”

**Answer:** Thank you. Done (Abstract, page 1 line 13).

**Background:**

1. Page 2, Line 8: Please change “have” to “has”

**Answer:** Thank you. Done.

2. Page 2, Line 11: Why is this group “heterogeneous”? Is aerobic capacity different in adolescents compared to young adult patients? Furthermore, one might argue that in clinical practice adolescents and young adults are the majority of the CF patients (i.e. the ones that are old enough to perform maximal-effort tests)

**Answer:** The study of Hebenstreit et al. identified that high levels of PA were associated with high aerobic capacity, but the results were obtained recruiting a CF group aged from 12 to 40 years. We used “heterogeneous” to underline that the CF subjects studied by Hebenstreit were adolescents, young adults and old adult.

The sentence has been changed in the revised manuscript and it was moved from the background to the discussion (Discussion, page 11 lines 4-8).

3. Page 3, line 4: As stated above, it needs to be more explicitly stated that the authors conduct this study to assess the possible utility of PA in substituting maximal CPET in aerobic assessment in CF.
Methods

1. Page 3, Line 21: Please delete repeated “had”.

   **Answer:** Thank you. Done.

2. Page 3, Line 24: Strictly speaking the control group is not age-matched but only “matched for age as possible”.

   **Answer:** Thank you. Done.

3. Page 3, Line 26: Please delete “at least 18 years of age”: since they are matched, they would be at least 18 years of age.

   **Answer:** Thank you. Done.

4. Page 4, Line 2: Please delete “that could contribute to breathlessness or exercise limitation”: this is a somewhat subjective comment; the reason for exclusion is obvious.

   **Answer:** Thank you. Done.

5. Page 4, Line 5-8: Please amend to: “This was a prospective case-control study. All subjects provided informed consent. The study was approved by the ethics committee of … Rome, Italy”

   **Answer:** Thank you. Done.

6. Page 4, Line 19: Please substitute “Experimental visits…for each subject” with “Assessment was conducted at the same place and time of day for all subjects”.

   **Answer:** Thank you. Done.


   **Answer:** Thank you. Done.

8. Page 4, Line 26: Do the authors report post-bronchodilator spirometry data?

   **Answer:** We reported baseline spirometry data.

9. Page 5, Line 2: Please correct “neural” to “neutral”?

   **Answer:** Hand-grip strength was removed as suggested by reviewers
10. Page 5, Line 5: Please delete “recommended”

**Answer:** Thank you. Done.

11. Page 5, Line 9: Please substitute “arterial” with “transcutaneous”. (Unless arterial samples were actually obtained)

**Answer:** Thank you. Done.

12. Page 5, Line 12: The authors mention “carbon dioxide production and end-tidal CO2 partial pressure”. Do these indices appear in the results?

**Answer:** V'O₂ and V'CO₂ are utilized for defining the LT.

13. Page 5, Line 15: please change “least” with “last”?

**Answer:** Thank you. Done.


**Answer:** Thank you. Done.

15. Page 5, Line 23: How is a “typical” day defined? Consider omitting “typical”

**Answer:** Thank you. Done.

16. Page 5, Line 23: Please clearly reference that this device has been validated in previous studies (your reference number 10).


17. Page 6, Line 14: What is “appropriate” training frequency: please consider rather giving an example of this level of activity as you did in the other categories: possibly running, cycling, swimming, rowing?

**Answer:** Thank you. Done. (Total body activity measurement, page 7 line 3).

18. You might consider not presenting all the indices that the device can measure but select the ones that aid your main points.
Answer: Thank you. Done. The table with PA variables has been modified. We selected the most important indices which are now in Table 1. The others indices are now presented as Supplement Table E1.

Statistics
1. Please consider justifying your sample size.

Answer: Thank you. The estimation of the calculation for sample size was done using a previous study by Troosters et al. [10] who showed a difference in VO₂ peak % predicted of 41 with a standard deviation of 13.62. We proposed that our effect size could be about half of that obtained by Troosters et al., therefore for 80% power and 5% significance and similar variability it would require a total 36 subjects in a ratio of 2:1. We therefore recruited 45 in total in a ratio of 2:1. We have now included this sentence in the methods section of the manuscript as the reviewer indicates (Study design, page 5 lines 10-15).

2. How was normality of distribution assessed?

Answer: Numeric data were presented as mean ± standard deviation (SD), for normally distributed data and comparisons done using the two sample independent t-test, while non-normal numeric data were presented as median (IQR) and comparisons done using the Mann-Whitney U test. (Statistical Analysis, page 7 lines 7-11).

Results
1. Page 7, line 6: I would omit the phrase “were well matched in terms of age, sex and body mass index”: strictly speaking they were not matched for age and sex, and BMI is not a parameter that would be intentionally “matched” by design, although it might be that there were no significant differences between the groups. (as in this study)

Answer: Thank you. Done.

2. Page 7, line 7: Are “the hand grip force and 6MWD” essential to this manuscript? Consider removing throughout.

Answer: The hand grip force and the 6MWT were not essential to this manuscript, so all the authors agreed to remove them from the results.

3. Page 7, Line 14-15: Please remove “None of the control…corticosteroids”. The authors don’t need to state this: in the methods they have stated that they excluded controls with any cardiorespiratory and neuromuscular pathology. In any case, if it were to be included, it should be in the Methods section.

Answer: Thank you. Done.

4. Page 7, line 19-21. Please delete “Compared with control subjects… compared to healthy subjects but…” All this information is included in the relevant table.
Answer: Thank you. Done.

5. Page 8, line 9-11: This is a general observation probably beyond the scope of this study. Consider removing.

Answer: Thank you. Done.


Answer: Thank you. Done.

7. Page 8. Line 21: The phrase “Single correlation analysis was conducted in CF patients” should be in Methods/Statistics. Furthermore, do the authors mean “bivariate” correlation analysis? If yes, which test was used?

Answer: Yes, we specified in the “statistical analysis” section that only for CF patients Spearman correlations were utilized to investigate associations such as whether any relationship existed between oxygen uptake both at lactic threshold and at peak exercise on the one hand and PA on the other. We have now included this sentence in the “statistical analysis” of the manuscript (page 7 lines 20-21) and we have removed it from the results session as the reviewer indicates.


Answer: Thank you. Done. VO2 expressed as ml/min.

9. Page 8. Line 24: Is the association of energy expenditure with VO2 at LT a novel finding? Should this be included in this manuscript? Consider removing.

Answer: Thank you. We agree with the reviewer. It has been removed.

10. Page 9. Line 2: Please note that these correlation coefficients are low. This should be discussed.

Answer: Thank you. PA of moderate intensity was related to VO2 uptake at LT, expressed as absolute (R=0.44, p=0.01) and relative to body weight (R=0.49, p=0.005). Physical activities above the threshold of moderate (>4.5 METS) and vigorous (>7.2 METS) intensity were also related to VO2 uptake at LT, expressed as absolute (r=0.45, p=0.01) and relative to body weight (r=0.46, p=0.009). Habitual PA at moderate intensity was positively related to VO2,peak, expressed relative to body weight, (R=0.49, p=0.005). Time spent in moderate and vigorous daily activities was correlated to VO2,peak both expressed as absolute and relative to body weight (R=0.41, p=0.02; R=0.51, p=0.003.) Using the same motion sensor for PA monitoring and the same METS levels, our findings were comparable to those of Troosters et al. who found that PA was moderately related to VO2,peak.

11. Overall the results need to be tailored down to the important ones that
highlight the study’s main point which is along the lines of “we suggest that CPET could be substituted by habitual PA in stable CF patients with mild-moderate lung disease”.

**Answer:** Thank you. Done.

Discussion  
1. Please accommodate major comments as highlighted above.

**Answer:** Thank you. Done. The major comments were highlighted in the Discussion as suggested by the reviewer. The sentences has been changed in the revised manuscript (Discussion, page 10 lines 10-25; page 11 lines 1-3, lines 16-18; page 12 lines 19-26; page 13 lines 10-13).

2. Page 10, line 1: The authors state that their group of adults with CF “were not engaged in specific home-based exercise training”. On page 11, line 13 they state that they were “encouraged to regular exercise”. So, it is not clear if and how much they exercised and what their baseline aerobic state was as this would certainly affect their measurements of CPET and PA.

**Answer:** Thank you. The sentence on page 11 has been changed in the revised manuscript as suggested by the reviewer as follow “We speculate that these results might follow our clinical practice in terms of patient education to enhance PA levels by explaining all the benefits associated with an active lifestyle.” Page 11, lines 18-20. As written above, patients and healthy controls were asked to continue any of their normal (habitual) activities. We did not provide any kind of training instructions or materials for the exercise, nor was their habitual physical activity reinforced by telephone contact. CF patients and controls wore the accelerometer for five typical days doing what they normally do. We have highlighted this concept both in the methods section of the manuscript and in the discussion (Total body activity measurement, page 6 lines 21-23; Discussion, page 12 lines 23-26).

3. Page 11. Lines 14-18: From “Adults with CF…and the same METS levels”. Consider removing as probably not relevant to the study’s main concluding points.

**Answer:** Thank you. Done.

4. Page 12, lines 6-7 and 15-18. On exercise capacity in CF and its relation with aerobic exercise it would be worth mentioning the effect of aerobic conditioning on respiratory muscle function.

**Answer:** Yes, there are few studies describing that quadriceps force was a significant contributor to the variance in 6MWT (Troosters, ERJ 2009). They also found that quadriceps force was related to V'O_{2,peak} when expressed in absolute values.
5. Page 12, lines 22-24: No need to mention this as a limitation: It has been clearly stated that “stable” patients with moderate lung disease were investigated.

**Answer:** Thank you. Done.

Table 2: Consider simplifying. What is “Active Energy expenditure”? Has this been explained?

**Answer:** Thank you. Done. We explained on the Methods section that the active energy expenditure is the number of calories per day due to a physical activity (Total body activity measurement, page 6 lines 17-18). We removed this variable from the main table and we putted it on the online supplement material.

Table 3: Consider simplifying: Please retain fewer parameters that support your basic conclusions.

**Answer:** Thank you. Done. We have delated the Table 3 and we have selected the most important PA indices which are now in Table 1. The other PA indices are now presented as Supplement Table E1.

Line 6: Authors state “unless otherwise stated” Is it otherwise stated anywhere?

**Answer:** Data are presented only as mean±SD or median (IQR).

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I have no competing interest with regards to this study.

**Reviewer 2:**

**Reviewer's report**

**Title:** Association of daily physical activity and exercise performance in adults with cystic fibrosis

**Version:** 1  **Date:** 4 September 2014

**Reviewer:** Narelle Cox

**Reviewer's report:**
The authors present a concise, and interesting, study of the relationship between
daily physical activity participation and submaximal and maximal exercise parameters in an exclusively adult cohort of individuals with Cystic Fibrosis. The study design is clear and the description of the study cohort thorough. The identification of why submaximal exercise data may be relevant in the clinical environment is well articulated, as is the recognition that the gender bias in recruited subjects may impose limitations on the generalizability of results.

Major compulsory revisions

Major Comments:

1. The authors do not appear to have taken the opportunity to discuss their results in the context of the similar results found in the study by Troosters et al (which the authors have noted in their introduction). Both studies report a moderate relationship between daily PA participation and exercise capacity in adults with CF. This comparison would be interesting for readers and may add weight to the findings of the present study.

**Answer:** Thank you. We agree with the reviewer and we have added some sentences in the discussion session (page 11 lines 4-11 and lines 20-26; page 12 lines 1-13). Specifically, there are only few studies in which physical activity levels were objectively assessed and compared with exercise capacity in an adult CF population. The largest study of Hebenstreit et al. (ERJ2006) identified that high levels of PA were associated with high aerobic capacity, but the results were obtained recruiting a heterogeneous CF group aged from 12 to 40 years, with a mean age of 20 years. The second study in which physical activity levels were assessed in adults with CF was done by Troosters et al. (ERJ 2009). They studied 20 CF patients with a mean age of 25 years and showed only a moderate relationship between PA and VO$_{2,peak}$ expressed as % pred. After correcting for covariates (FEV1, BMI, sex) PA only tended to explain further variance in VO$_{2,peak}$.

We conducted a study in 30 adults CF patients with 33 years old and we confirmed that that daily PA, particularly of moderate intensity or greater, correlated with measurements of exercise tolerance.

Also, the CF population examined by Troosters et al. revealed that the number of steps, PA at mild and vigorous intensity were not significantly different in patients compared with healthy subjects. Only activities at moderate intensity were reduced in patients with CF. Conversely in our study, we found no differences between CF and controls in any of the PA variables measured. Using the same motion sensor for PA monitoring and the same METS levels, our findings are comparable to those of Troosters et al. Eur Respir J 2009 for the number of steps (9160 versus 9398) and time spent in mild and moderate PA (13 minutes in our study versus 14.8 reported by Troosters et al.). Vigorous PA was greater in Troosters’s study (1 min versus 4.2).

Regarding PA levels of the healthy control group, our population seems to be more sedentary than Troosters’s control group. We highlighted as a limit of the study that our healthy controls are disease-free but possibly more sedentary, even if they were recruited from staff and colleagues of our hospital and they were physiotherapists, students and doctors (Discussion page 13 lines 21-26).

2. Discussion – Paragraph 5
I would encourage the authors to revisit comments indicating that adults with CF have good adherence to recommendations regarding activity behaviour. Neither of the references provided were studies of compliance with physical activity recommendations. In contrast studies of adherence report very variable (and low) rates to activity and exercise (e.g. Shepherd et al 1990; White et al 2007). Is it possible other factors contribute to the levels of PA participation? For example, that the CF patients in the present study did not undertake fulltime work and as such had more opportunity to undertake physical activity (particularly mild intensity)?

**Answer:** Thank you. Adults with CF were able to accomplish their activities of daily living as much as age-matched healthy controls with no differences in terms of PA intensity levels between the two groups. The sentence has been changed in the revised manuscript (Results, page 8 lines 16-19). We speculate that these results might follow our clinical practice in terms of patient education to enhance PA levels by explaining the benefits associated with an active lifestyle. The sentence has been changed in the revised manuscript (Discussion, page 11 lines 16-20). All patients undertake fulltime work and the age-matched healthy controls were recruited from staff and colleagues of the same hospital, so they are fulltime workers.

**Minor essential revisions**

3. 6 minute walk test (6MWT) may be a more accurate description in the abstract than 6MWD.

**Answer:** Thank you. As suggested by reviewers the 6MWT was removed from the manuscript.

4. The authors may wish to consider a paragraph break in the background after the reference to Troosters et al regarding moderate association between PA participation and exercise capacity, and before the information relating to PA participation by adults with CF compared to healthy peers to differentiate these two concepts.

**Answer:** Thank you. Done.

5. Methods – Subjects
Consider starting paragraph two with ‘fifteen’ rather than a numerical value.

**Answer:** Thank you. Done.

6. Methods – Study design
Could the authors indicate if the order of exercise tests was randomized?

**Answer:** Experimental visits and the order of exercise tests were conducted at the same place and at the same time of day for each subject. This information was highlighted in the Methods (Study design, page 5 lines 8-9).

7. Methods – Procedures
Could the authors clarify the number of 6MWTs which participants undertook.

**Answer:** CF patients and controls performed the 6MWT test in accordance with ATS recommendations with two additional practice walks at the initial assessment.

8. Statistical analysis
Line 3 – the abbreviation CF can replace cystic fibrosis.

**Answer:** Thank you. Done.

9. Discussion – Paragraph 6:
Consider clarifying the statement that ‘factors other than PA levels might influence exercise capacity in CF’ to influence performance on 6MWT as the present statement seems contradictory to your reported results of a relationship between PA and exercise capacity.

**Answer:** As suggested by reviewers the 6MWT was removed from the manuscript.

Table 1 - there are spelling errors in the title

**Answer:** Corrected. Thank you.

Table 2: can the authors clarify if PA participation was normally distributed? If not, would activity time be better presented as median (IQR)?

**Answer:** Thank you. The new statistical analysis was done. PA data which were not normally distributed are reported as median (interquartile range). Page 7, lines 8-11.

Discretionary revisions
1. Discussion – Paragraph 3:
I too am intrigued by the possibility that changes in daily PA may indicate a decline in exercise tolerance, and look forward to the day we can work this out!

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests.

**Reviewer 3**

**Reviewer's report**

**Title:** Association of daily physical activity and exercise performance in adults with cystic fibrosis
1. The primary research question needs further clarification. The authors are investigating (1) the relationship between physical activity and exercise parameters (submaximal and maximal intensities), (2) the difference between cystic fibrosis (CF) and healthy controls in physical activity and exercise capacity parameters, (3) within group changes in physiological parameters during cardiopulmonary exercise testing (CPET) from rest to peak exercise. Although the title of the manuscript implies that the correlation between physical activity and exercise tolerance test results is the main focus, the exploration of the other aforementioned research questions causes confusion as to the authors’ primary objective in this study.

Answer: Thank you. As suggested, we modified the introduction and we clearly wrote that the purpose of this study was twofold. First, we aimed to examine the relationship between daily PA and aerobic fitness both at submaximal and maximal levels in clinically stable adults with CF. Specifically, we were interested in evaluating PA as a surrogate of CPET in the assessment of exercise capacity in CF. In addition to this primary aim, we wished to compare both daily PA levels and responses to CPET of adult CF patients and healthy controls. In analyzing the possible positive effect of habitual PA in CF, we sought to evaluate the parameters obtained at submaximal level of exercise in CF and in healthy control peers. Background page 3 lines 22-26; page 4 lines 1-3.

2. The findings of this study show an association between physical activity parameters and various peak and submaximal CPET parameters; however, the authors report no association between the 6 minute walk test and physical activity. More discussion on these conflicting findings would have been helpful (ie possible reasons why physical activity correlates with submaximal intensity parameters during CPET but not a standard submaximal exercise test such as the 6 minute walk test; could there be possible limitations in the study design that may have resulted in these findings?).

Answer: Thank you. Even increasing our simple size (30 adults with CF), we confirmed that 6MWT appeared still similar between patients and controls. Despite this positive result, the 6MWT was not related to the PA outcomes. Our data is in line with recent study, where the authors reported no correlation between 6MWT and PA levels in 20 adults with CF [Troosters et al. ERJ 2009]. We also found that the 6MWT (which is a submaximal exercise test) did not correlate with physiological responses to submaximal CPET. Inversely, PA variables did correlate with both submaximal and maximal responses to CPET. So, this single study provide evidence that objective PA assessment should be preferred in evaluating aerobic fitness in adults with CF. Further
research in larger CF patients group is needed to enhance the understanding of the 6MWT role. The 6MWT results were removed from the manuscript as suggested by reviewers.

3. Concern over the performance of a peak exercise test on the same day as the 6 minute walk test. Depending on the order of testing, the performance of one test may affect the results obtained for the subsequently performed test. Information regarding the time between both exercise tests would be helpful to determine if participants had an adequate amount of recovery time before the performance of the second exercise test. It may have been more appropriate to conduct the exercise test on separate study days.

   Answer: Thank you. Assessment was conducted at the same place and time of day for all subjects. Specifically, CPET was conducted 30 min after the 6MWT.

4. An explanation/calculation for the chosen sample size is missing. Please address.

5. Answer: Thank you. Done. A description of the sample size calculation is added on the methods section in our new manuscript (Study Design, page 5 lines 10-15).

6. The inclusion of a control group for the purpose of comparing exercise tolerance and physical activity between groups in this study seems to be inappropriate if the primary objective of the study is to determine the relationship between physical activity and exercise tolerance. Please clarify the rationale for including the control group and what value their inclusion adds to this study. Would have been interesting to see the relationship between physical activity and exercise tolerance in the control group and compare those findings with the CF group.

   Answer: The rationale for including the control group was because the second aim of this study was to compare both daily PA levels and CPET responses of adult CF patients and healthy controls. Even if few studies have compared daily PA participation in adult CF patients and in healthy subjects (Troosters, ERJ 2009; Savi, Resp Med 2013; Rasekaba et al. J Cystic Fibrosis 2013), it still remains unclear whether CF adults have different habitual activities compared with healthy peers. So, in order to clarify the discrepancies in the current literature we conducted this study.

   Also, despite our knowledge about the beneficial effects of a supervised exercise intervention on submaximal and maximal oxygen uptake in CF [Gruber, Eur Respir J 2011; Gruber, J Pediatr 2011], there have been no studies examining the effect of habitual daily PA on submaximal workload parameters in CF. In analyzing the possible positive effect of habitual PA in CF, we sought to evaluate the parameters obtained at submaximal level of exercise in CF and in healthy control peers.

   The correlations between CPET parameters (at least for the V'O2 response) at submaximal level of exercise and PA and their similarity with those observed in our healthy controls, made us assume that habitual PA may positively affect the submaximal exercise parameters in adults with CF (Discussion page 13 lines 10-13).
Minor Essential Revisions:

7. Would have been helpful if validity and reliability of the SenseWearTM activity monitor was discussed (ie references to previous validation studies). Has this monitor been validated for use in a CF population? Please reference the previous validation studies of this activity monitor in a CF or similar chronic lung disease population.

**Answer:** Thank you. Done. References about the validity of the SenseWear Armband in CF were added to the manuscript [Dwyer T, Respir Med 2009; Cox NS, Journal of Cystic Fibrosis 2014]. We have now included this sentence in the methods section of the manuscript as the reviewer indicates (Total body measurement, page 6 lines 10-11).

8. In the Discussion section (paragraph 2), the authors state “VO2 at which lactate starts to increase closely reflects the fitness of an individual and can increase with a training program”. This statement needs a reference for evidence, especially previous studies which have shown an increased VO2 at which lactate accumulates with physical training programs in CF.

**Answer:** Thank you. Done. The reference was written (Gruber, Eur Respir J 2011).

9. Borg leg and dyspnea findings during CPET were reported in paragraph 3 of the Results section (paragraph one of the Physiological responses to CPET); however these measurements were not discussed in Methods section as one of the CPET measurements. Please add it to the list of measurements during CPET in Methods section. Also, Borg scales require a reference to the research literature from which the grading scheme originated.

**Answer:** Thank you. Done. We added it to the list of measurements during CPET in Methods section (page 6 lines 1-3). The reference was also reported (Borg GA. Med Sci Sports Exerc 1982).

10. Grammar – Background section (paragraph 2 – line 23): “The relationship of adult CF patients to healthy subjects in terms of daily PA to date it has not been well established ...”. This statement seems awkward, please consider rewording.

**Answer:** Thank you. Done. We rewrote as “it remains unclear whether CF adults have different habitual activities compared with healthy peers and in order to clarify the discrepancies in the current literature further studies are needed.” Background, page 3 lines 15-17.

11. Grammar – Procedures section (paragraph 2 – line 15): “Exercise variables were measured and averaged over the least 10 seconds...”. Did the authors mean to say “last 10 seconds”?

**Answer:** yes

Discretionary Revisions:
Reviewer 4

Reviewer's report:

Title: Association of daily physical activity and exercise performance in adults with cystic fibrosis

Version: 1  Date: 14 September 2014

Reviewer: Michelle Murray

Reviewer's report:

Discretionary Revisions only

This is a well thought out prospective study and even though as the authors acknowledge is in a small group attempts to address the relationship between sub maximal exercise and physical activity in CF patients with mild to moderate FEV1. Can the authors suggest why there may not be a relationship between physical activity and 6MWT. In the study of the particular genotype of pts was there a difference between del 508 homozygous in comparison to others studied?

Answer: Thank you. Even though we increased our simple size and studied 30 adults with CF, we found that 6MWT was still similar between patients and controls. Despite this positive result, the 6MWT was not related to the PA outcomes. Our data is in line with recent study, where the authors reported no correlation between 6MWT and PA levels in 20 adults with CF [Troosters]. We also found that the 6MWT (which is a submaximal exercise test) did not correlate with physiological responses to submaximal CPET. Inversely, PA variables did correlate with both submaximal and maximal responses to CPET. So, this single study provide evidence that objective PA assessment should be preferred in evaluating aerobic fitness in adults with CF. Further research in larger CF patients group is needed to enhance the understanding of the 6MWT role.
Reviewer 5:

Reviewer's report:

Title: Association of daily physical activity and exercise performance in adults with cystic fibrosis

Version: 1 Date: 17 September 2014

Reviewer: David Serisier

Reviewer's report:

This was a prospective comparison of 16 CF patients and 15 healthy controls to assess relationships between daily physical activity (assessed by SenseWear armband) and both maximal incremental CPET by cycle ergometry and 6MWT. The main findings in this small, open study were that firstly, CF patients manifested higher overall daily physical activity than controls, in spite of significantly poorer lung function. Secondly, during CPET, controls achieved higher total workload and CF patients did not appear to achieve exercise limitation during CPET (in contrast to controls, who did exercise maximally). Thirdly, in CF patients statistically significant correlations (although mostly borderline statistically significant and without Bonferroni corrections) were observed between the daily time spent in moderate and vigorous physical activity and a number of absolute (but not %predicted) CPET measures including VO2 and workload. Overall in this paper I found it difficult to follow a logical progression from identification of a specific problem or knowledge gap through to methods and results that addressed this and then a discussion that contextualized the findings. The rationale for the underpinnings for this study need to be better explained, the evidence gap that this study addresses needs to be more clearly elucidated. Furthermore, the data suggest significant confounding factors to the results obtained, with suggestion that the CF subjects here were motivated ‘exercisers’ while the healthy controls were somewhat sedentary. Finally, some of the interpretations of the data do not seem to accurately reflect the results.

Major Comments:

1. The paper frequently refers to the investigation of relationships between physical activity and submaximal (and maximal) exercise tolerance. However, there was no submaximal exercise testing performed, and the authors instead are using
measures of physical fitness at the lactate threshold. This might be an acceptable surrogate for submaximal fitness but I am not sure it necessarily represents submaximal exercise tolerance per se.

Answer: Thank you. We agree with the reviewer that submaximal exercise related data (also known as lactate threshold-LT measures) is more reflective of exercise fitness and may also be useful in the evaluation of patients who may not be able or willing to provide a maximal effort, especially when maximal exercise tolerance is limited by ventilatory factors [Wasserman K, Am Rev Respir Dis 1984.]. So the term “tolerance” has been replaced by the term “fitness” (Abstract, page 1 line 7; Background, page 3 line 23).

2. A) The primary hypotheses of the study was that ‘daily PA and parameters obtained at submaximal exercise would be similar to healthy controls’ (I am assuming this is meant to refer to similarity between CF and normal controls). However, no true submaximal exercise test was performed.

Answer: Thank you. For clarity, in this paper “Healthy controls” = “normal controls” (without recognised diseases). The sentence was changed as suggested by the reviewer as followed: “In addition to this primary aim, we wished to compare both daily PA levels and responses to CPET of adult CF patients and healthy controls. In analyzing the possible positive effect of habitual PA in CF, we sought to evaluate the parameters obtained at submaximal level of exercise in CF and in healthy control peers”. Page 3 lines 25-26, page 4 lines 1-3.

We agree with the reviewer that a submaximal test would be more appropriate for comparison with PA in order to investigate the possible effects of the latter on exercise tolerance. Nevertheless, considering parameters at submaximal level of exercise (i.e. at LT) was done with the intention to assess patients state of fitness (and not exercise tolerance) at submaximal level of exercise intensity, which in turn should reflect more closely normal daily activities. The correlations between CPET parameters (at least for the VO2 response) at submaximal level of exercise and PA and their similarity with those observed in our healthy controls, made us assume that habitual PA may positively affect the submaximal exercise parameters in adults with CF (Discussion page 13 lines 10-13).

2.B) Secondly, attempting to answer the first hypothesis with any confidence would require much larger patient numbers than present in the current study (given the multiple possible contributors to exercise tolerance in individuals both with and without CF) and no powering data are presented.

Answer: In the new manuscript, the estimation of the calculation for sample size was done using a previous study by Troosters et al. [ERJ 2009] who showed a difference in VO2 peak % predicted of 41 with a standard deviation of 13.62. We proposed that our effect size could be about half of that obtained by Troosters et al. therefore for 80%
power and 5% significance and similar variability it would require a total 36 subjects in a ratio of 2:1. We therefore recruited 45 in total in a ratio of 2:1. We have now included this sentence in the methods section of the manuscript as the reviewer indicates (Study design, page 5 lines 10-15).

To investigate whether exercise capacity was different between CF patients and healthy controls, after correcting for potential confounding covariates like age, BMI, FEV₁ and gender, the latest square means were computed for the dependent variables using a generalized linear models procedure. These analyses were done for variables that were normally distributed. A pragmatic decision was taken to set the significance level at $p \leq 0.01$ to correct for multiple comparisons. We have now included this sentence in the methods section of the manuscript as the reviewer indicates (Statistical analysis, page 7 lines 20-25, page 8 lines 1-2).

2.C) Thirdly, I do not understand why the parameters obtained at the lactate threshold would be expected to be similar between subjects with and without lung disease? There is no argument to support such a hypothesis – given that significant lung disease should result in the AT being reached earlier following exercise onset (and hence VO₂ and W should be lower in subjects with lung disease), subjects with CF should be expected to have similar parameters only if they are physically fitter (in which case their daily PA should be higher) – ie how is the rationale behind the first hypothesis consistent with the rationale behind the second hypothesis? Although there is discussion in the introduction behind PA and exercise testing general, there is little convincing argument in the introduction that leads to the study hypotheses and study design.

**Answer:** Thank you. We rewrote the introduction and we have added sentences for clarity as suggested by the reviewer. Background, page 3 lines 8-21. “Few studies have compared daily PA participation in adult CF patients and in healthy subjects. The CF population examined by Troosters et al. (ERJ 2009) revealed less time spent in moderately intense PA compared with healthy controls. Conversely, our recent study demonstrated that the time spent in daily PA at different intensities was similar in adults with CF and age-matched healthy controls [Savi D, Resp Med 2013]. Similarly, Rasekaba et al. showed that moderate or vigorous habitual physical activities measured using a validated questionnaire were similar between CF adults and controls [J Cyst Fibrosis 2013]. So, it remains unclear whether CF adults have different habitual activities compared with healthy peers and in order to clarify the discrepancies in the current literature we conducted this study. Also, despite our knowledge about the beneficial effects of a supervised exercise intervention on submaximal and maximal oxygen uptake in CF (Gruber W, *Eur Respir J* 2011; Gruber W, *J Pediatr* 2011), there have been no studies examining the effect of habitual daily PA on submaximal workload parameters in CF."

We also rewrote the sentence “As our second aim, we wished to compare both daily PA levels and responses to CPET of adult CF patients and healthy controls. In
analyzing the possible positive effect of habitual PA in CF, we sought to evaluate the parameters obtained at submaximal level of exercise in CF and in healthy control peers.” Background page 3 lines 25-26, page 4 lines 1-3.

The results of the study evidenced similar levels of daily PA between CF and healthy controls. The sentence has been changed in the revised manuscript (Results, page 8 lines 16-19). CF patients and controls reached their lactic threshold (LT) at a similar \( V'O_2 \) and work rate; conversely, they stopped exercise at a lower peak \( V'O_2 \), work rate and HR than healthy controls. So our study demonstrated that, despite reduced exercise tolerance, CF patients presented \( V'O_2 \) responses to CPET comparable to healthy controls and reached their LT at a work rate and \( O_2 \) uptake similar to that of healthy controls. This is important if we consider that the \( V'O_2 \) at which lactate starts to increase closely reflects the fitness of an individual and can increase with a training program (W. Gruber, ERJ 2011).

The novelty of the present study was that we observed these CPET responses in a group of adults with CF who were not engaged in specific home-based exercise training but were generally active as part of their daily lifestyle. This study was conducted recruiting adults CF patients consecutively from the CF outpatient clinic and healthy control subjects from staff and colleagues of the same hospital by invitation. CF patients and healthy controls were asked to continue their habitual daily activities. We underlined that they were not engaged in a specific home exercise training programme. We did not provide any kind of training instructions or materials for the exercise, nor was their habitual physical activity reinforced by telephone contact. CF patients and controls wore the accelerometer for five typical days doing what they normally do.

We have highlighted this concept both in the methods section of the manuscript and in the discussion (Total body activity measurement, page 6 lines 21-23; Discussion, page 12 lines 23-26).

The correlations between CPET parameters (at least for the \( V'O_2 \) response) at submaximal level of exercise and PA and their similarity with those observed in our healthy controls, made us assume that habitual PA may positively affect the submaximal exercise parameters in adults with CF. This concept was added in the Discussion on page 13 lines 10-13.

3. Further to 2 above, given that there were significant differences between the CF and normal control subjects for PA (CF subjects undertook significantly more daily physical activity and higher average Mets than the normal controls; they also spent an average of more than double the daily time spent in moderate PA, although this was reportedly not statistically significantly different), there is a lack of matching between the 2 groups for this factor which would be expected to predict AT and the parameters measured at AT. It is likely that this is why CF subjects had similar such results to controls – in spite of their lung disease they are more physically fit.

Answer: Thank you. These previous results were due to both the small number of CF subjects and the PA parameters were not normally distributed. In our revision, with a larger CF population studied (30 CF patients) and with a new statistical analysis, we
found no differences in daily PA between CF and healthy controls. The sentence has been changed in the revised manuscript (Results, page 8 lines 16-19).

4. Further to 3, the CF subjects in this study appear relatively motivated/physically fit, while the normal controls do not – there is therefore the suggestion of imbalance in the physical activity motivation of the 2 comparator groups/selection bias. Ideally, such important variables would be controlled for between the 2 groups.

**Answer:** Thank you. We studied daily PA in 30 adults with CF, which is a bigger population than previous studies *(Troosters et al. ERJ 2009; Ward et al. Resp Med 2013)*. Using the same motion sensor for PA monitoring and the same METS levels, our findings were comparable to those of Ward *et al.* who investigated 24 adults with CF after one month post-discharge, for the mean time spent doing mild, and vigorous PA (mild: 159 minutes in our study vs. 163 min in Ward’s study; vigorous: 1 min in our study and 3 min in Ward’s study) despite moderate PA being lower in our study 13 vs 43 min. Our findings are also comparable to those of Troosters *et al.* (*Eur Respir J 2009*) who investigated 20 stable CF adult patients for the number of steps (9160 vs. 9398), times spent in mild and moderate PA (13 minutes in our study vs. 14.8 reported by Troosters *et al.*). Vigorous PA was greater in Troosters’s study (1 min in our study vs. 4.2).

Regarding PA levels of the healthy control group, our population seems to be more sedentary than Troosters’s control group. Despite similar number of steps (10591 in our study vs. 10281 in Troosters’s study), PA at moderate and vigorous intensity were lower (moderate PA 11 min in our study vs. 34 min and vigorous PA 1 min vs. 9 min in Troosters’s study).

We have now included a new sentence in the discussion section of the manuscript (Discussion, page 11 lines 20-26, page 12 lines 1-13).

We have highlighted as a limit of the study that our healthy controls are disease-free but possibly more sedentary, even if they were recruited from staff and colleagues of our hospital and they were physiotherapists, students and doctors. Unfortunately, it was technically difficult to recruit a different control group at the time we conducted the study. It is possible that the reasons for the differences in PA may reflect cultural differences, subject motivation to participate in the study or an issue of sampling error. We have now included this sentence in the discussion section of the manuscript as the reviewer indicates (Discussion, page 13 lines 21-26).

5. The CF subjects in this study did not exercise to limitation. This should not affect the AT findings, but clearly makes it hard to interpret the parameters measured at ‘maximal’ exercise.

**Answer:** Exercise intolerance is a clear hallmark of CF *(Orenstein DM, Curr Opin Pulm Med 2005)*. Our findings are comparable to those of Troosters *et al.* (*Eur Respir J 2009*) who studied 64 stable CF adult patients. Specifically, parameters at peak of incremental CPET were: Work rate: our study 168±36; Troosters’s study 155±57
V'O$_2$%predicted maximum: our study 76 ±13; Troosters's study 71±18
V'O$_2$.ml/min/kg: our study 29 ±5; Troosters’s study 30.2±9.7
HR, beats·min$^{-1}$: our study 155± 12; Troosters’s study 176±16
%predicted maximum: our study 83±7.5; Troosters’s study 90±7.2

6. Table 2. It does not make sense to me that CF subjects undertook more physical activity, slept/ lay down less, had more active energy expenditure, more steps, etc, however had very similar total energy expenditure to the normal controls??

One would expect that the non-recorded components of energy expenditure here (eg resting energy expenditure, energy expenditure from coughing and chest clearance activities, etc) would be substantially higher in CF than controls, as has been shown previously and therefore ‘adding’ all these components should result in substantially higher total energy expenditure in the CF group. Is there an error in the data reported here or some problem with the sensewear technology used in this study? Why this discrepancy?

**Answer:** Thank you. Studies performed in CF have shown that the hypersalinity of sweat does not affect the accuracy of the SenseWear Armband estimation of Energy Expenditure and also have demonstrated that the device provides a reasonably accurate estimate of physical activity in the free-living environment [Dwyer T, Respir Med 2009; Cox NS, Journal of Cystic Fibrosis 2014]. We reported these studies as References 29 and 30.

Increasing the simple size with new statistical analysis, no statistically differences were found between CF and controls. The sentence has been changed in the revised manuscript (Results, page 8 lines 16-19).

7. The CF subjects were mostly taking bronchodilators while controls clearly were not – how was the use of these regulated during the evaluation periods of the study and how would this have affected the findings?

**Answer:** Thank you. All patients were instructed to take all of their maintenance medications as usual.

8. Interpretations – results page 7 (‘compared with control subjects, patients with CF had similar values in daily life PA’) – I do not comprehend how such an interpretation can be made with the data presented.

**Answer:** Thank you. Increasing the simple size with new statistical analysis, no statistically differences were found between CF and controls. The sentence has been changed in the revised manuscript (Results, page 8 lines 16-19).

9. Reporting of univariate correlations should generally be made with caution, but particularly in a sample size of only 16 subjects, where multiple correlations seem to have been undertaken (but only the positive ones have been reported), and there has been no adjustment for multiple testing.
**Answer:** Thank you. We did not apply any specific test like the Bonferonni correction to adjust the p values for our comparisons, however we took a pragmatic decision to set the significance level at \( p \leq 0.01 \) to correct for multiple comparisons. The sentence was added in the revised manuscript (Statistical Analysis, page 8 lines 1-2).

10. I doubt that this study is powered to demonstrate equivalence, between CF and control subjects, for the 2 primary hypotheses (altho no powering calculations are presented or considered). Before the authors can claim that CF subjects have similar VO2 at AT to controls, they would need to provide convincing powering calculations.

**Answer:** Thank you. The sample size for our study was large enough to detect differences between CF patients and controls even at 90% power. A previous study by Troosters et al. showed a difference in peak VO2 of 41 with a standard deviation of 13.62. We proposed that our effect size could be about half of that obtained by Trooster, therefore for 80% power and 5% significance and similar variability would require a total 36 subjects in a ratio of 2:1, we therefore recruited 45 in total in a ratio of 2:1. We have now included this sentence in the methods section of the manuscript as the reviewer indicates (Study design, page 5 lines 10-15).

11. No data are presented upon the exercise ordinarily undertaken by subjects in the study, except to state in discussion that they ‘were not engaged in specific home-based exercise training’. Why did the CF patients have greater daily PA then?

**Answer:** Thank you. CF patients and healthy controls were asked to continue their habitual daily activities. We underlined that they were not engaged in a specific home exercise training programme. We did not provide any kind of training instructions or materials for the exercise, nor was their habitual physical activity reinforced by telephone contact. CF patients and controls wore the accelerometer for five typical days doing what they normally do. We have highlighted this concept both in the methods section of the manuscript and in the discussion (Total body activity measurement, page 6 lines 21-23; Discussion, page 12 lines 23-26).

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
i declare that i have no competing interests.
We finally inform you that:

- the present manuscript, or part of it, has neither been published nor is currently under consideration for publication by any other journal.
- Pierantonio Laveneziana and Paolo Palange have equally contributed to the manuscript and are both last authors.
- Daniela Savi is a PhD fellow in “Biotechnology applied to Clinical Medicine”, Sapienza University of Rome, Rome, Italy.

Kind regards,

Dr. Daniela Savi