

## Peer Review File

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### Review comments 1

This study investigated the association between fat oxidation rates and exercise intensity across wide ranges of age and ASM/Wt. This is an ambitious goal but the study suffered several drawbacks in study design and the interpretation of the results. The selection for references also needs to be improved.

**Reply: We would like to thank the reviewer for the encouraging words. We have endeavored to satisfactorily meet recommendations of revisions.**

1. This study used absolute workload, rather than relative workload, such as % Wmax, making the comparison across wide ranges of age and ASM/Wt difficult, if not impossible.

**Reply: Thank you for identifying this limitation. The study was designed specifically to encompass a large age range as we were researching the effect of age on the data collected. You raise a good point regarding the difficulty in direct comparisons, given the likely differing levels of health and activity. We have thus expressed this limitation in our discussion section.**

**Changes in the text: page 14, line 324-331**

2. The authors did not explain why fat oxidation rate was correlated with ASM/Wt and age only at 10 and 20 W, but not in higher workloads. In fact, even 50 W is relatively easy for younger and middle-aged subjects.

**Reply: We appreciate the reviewer's opinion. 50W is a sizeable workload when considering the race and age of our study participants. However, we do appreciate your viewpoint and will consider higher workloads in future studies.**

3. Line 68-69: reference 6, a review, did not specifically mention the infiltration of lipid in the

ageing muscles. Please cite other references. Reference 7, a review, did not specifically link the accumulation of lipids in the ageing muscles to insulin resistance. The authors need to cite more specific references to support their point that lipid accumulation in the muscles leads to insulin resistance in the ageing people.

**Reply:** Thank you for your kind reminds. We have amended the references in question.

**Changes in the text:** page 3, line 69-70, reference 6-7

4. Line 70: Increasing fat oxidation rates (FORs) is likely to be related to reduced insulin resistance is too vague. In addition, reference 8 suggested that *mcd(-/-)* mice exhibit reduced (not increased) rates of fat catabolism and were resistant to diet-induced glucose intolerance.

**Reply:** Thank you for your careful check and kind reminds. We are sorry for our inaccurate description. We have amended the description and corrected reference 8.

**Changes in the text:** page 3, line 71-73, reference 8

5. Line 75-77: These statements are irrelevant to substrate utilization.

**Reply:** We appreciate the reviewer's opinion. We have removed the statements which are irrelevant to substrate utilization and improved this paragraph.

**Changes in the text:** page 3, line 78

6. Line 103: Why the authors decided to measure thyroid hormones but not insulin? Insulin resistance seemed to be one of the major interests in this manuscript.

**Reply:** We would like to thank the reviewer for their comment. There is a negative correlation between insulin and ASM/Wt ( $r = -0.228$ ,  $P=0.018$ ), as you can see in the table 1.

**Changes in the text:** page 8, line 188-189

7. Line 143-144:  $VO_{2peak}$ .

**Reply:** Thank you for your careful check and kind reminds. We have amended the sentence.

**Changes in the text:** page 7, line 163

8. Line 160-161: reference 15 did not investigate the relationship between appendicular skeletal muscle mass/Weight (ASM/Wt) and insulin resistance. Please select another

reference.

**Reply:** Thank you for your careful check and kind reminds. We have amended the reference.

**Changes in the text:** page 8, line 184, reference 15

9. Line 163-164: Legs lean masses 163 decreased much more than arms lean masses during ageing (legs:  $r = -0.411$ , arms:  $r = -0.345$ ). The statement is probably based on the difference between the 2 correlation coefficients. However, the authors did not compare if these 2 correlation coefficients are indeed significantly different.

**Reply:** We would like to thank the reviewer for the constructive suggestions. We indeed did not compare these 2 correlation coefficients. Therefore, we have removed the problematic description.

**Changes in the text:** page 8, line 186

10. Line 176-177: The results suggest that individuals with strengthened muscle mass have an increased capacity to oxidize fat during low-intensity exercise.

**Reply:** We appreciate the reviewer's opinions. We have removed the sentence in question.

**Changes in the text:** page 9, line 206

11. Line 206-207: Chinese men with decreased muscle mass might need higher exercise intensity to achieve Fatmax. This statement is not directly supported by the results. If the authors want to make this statement, it is better to be moved to Discussion.

**Reply:** We appreciate the reviewer's opinions. We have removed the sentence in question.

**Changes in the text:** page 10, line 241

12. Legend in figure S2: correction should be 'correlation'.

**Reply:** Thank you for your kind reminds. We have amended it.

**Changes in the text:** Supporting materials

The study in question has aimed to assess the correlation between fat oxidation rates of skeletal muscle and exercise with aging. The strengths of this study are in its demographic base and the relatively large subject group assessed. I found this study to be both interesting and relevant for this journal, however there are a number of issues I feel would greatly improve the quality of the article.

#### Methods

1. Please include relevant references for the methods section, such as for your "Weir equation".

**Reply:** We would like to thank the reviewer's advice. We have added the references to back up this statement.

**Changes in the text:** see page 6, line 148, reference 14

#### Results

2. In the result," ASM/Wt was found to be positively related with FT3", it is worth considering reading Bloise et al. 2018 - "Thyroid Hormones Play Role in Sarcopenia and Myopathies".

**Reply:** We would like to thank the reviewer for this comment. We have included the Bloise et al. 2018 reference to highlight our data as consistent with a previous study as follows: *"Meanwhile, ASM/Wt was found to be positively related with FT3. There is increasing evidence that thyroid hormones play a role in muscle loss process (36), for example, in one study hyperthyroid mice exhibited higher muscle fatigue (37)."*

**Changes in the text:** see page 14, line 320-323

3. Were all 224 subjects included in all resulting outcomes?

**Reply:** We would like to thank the reviewer for their question. Not all 224 subjects were included in all resulting outcomes. As noted in our Methods section, the incremental exercise test data was obtained for only 121 recruited men of the original 224 subjects pool.

**Changes in the text:** see page 5, line 115-117

#### Discussion

4. In the discussion, the author state "however, the implied relationship between energy expenditure and muscle mass has still not been clearly established" - please check for references and revise.

**Reply:** Thank you for your comment. We have removed the sentence in question and have replaced it with discussion about available evidence in the literature on this topic as follows: *“Some studies have shown that more muscle mass increases REE(21), but other studies have opposite finding, for example, a study did not demonstrate a low REE in Duchenne Muscular Dystrophy (DMD) males(22).”*

**Changes in the text:** see page 11, line 257-259

5. "In general, the fractional contribution of CHO to the fuel supply is small at low intensity and rises with the exercise intensity increase." - Back it up with a reference.

**Reply:** Thank you for your reminder, we have added the reference to back up this statement.

11.van Loon LJ, Greenhaff PL, Constantin-Teodosiu D, Saris WH, Wagenmakers AJ. The effects of increasing exercise intensity on muscle fuel utilisation in humans. J Physiol 2001; 536: 295-304

**Changes in the text:** see page 4, line 90, reference 11

6. There are three red punctuation marks in the middle of a sentence in the discussion.

**Reply:** Thank you for noticing this, we are sorry for missing this prior to submission. We have amended the sentence in question, removing the three punctuation marks and replacing it with “more muscle mass”.

**Changes in the text:** see page 12, line 285

### **Review comments 3**

This manuscript is entitled “fat utilization of muscle decreased during aging when light intensity exercise”. 224 health aged subjects were recruited into this study, body composition was measured by DXA, exercise challenge were performed by ergometer cycle and determined energy expenditure using indirect calorimetry.

The results showed positive relationship between ASM/WT and FORs. However, FORs did

not show any correlation between ASM/WT and Fatmax. Finally, this study suggested that old men with decreased muscle mass need more strenuous exercise to achieve Fat max. However, there are some questions/suggestions need to be clarified.

1. All subjects were collected blood samples for analysis fasting levels of glycated hemoglobin, cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein. However, we could not find these blood analysis item in biochemical analysis section just only mentioned about glucose, insulin, triglycerides, cholesterol, FT3, FT4, and TSH.

**Reply:** We would like to thank the reviewer for their question. We have amended the results section to include both significant and non-significant data as follows:

*“However, there was no statistical association between ASM/Wt and fasting glucose, HDL-C, HAc1%, 25(OH)VD and thyroid function including FT3, FT4 and TSH.”*

**Changes in the text:** page 8, line 189-191

2. In the method “REE calculated using the Weir equation...”, the authors need to cite the reference about Weir equation please.

**Reply:** We have included the appropriate reference for the Weir equation.

Mehta NM, Smallwood CD, Joosten KF, Hulst JM, Tasker RC, Duggan CP. Accuracy of a simplified equation for energy expenditure based on bedside volumetric carbon dioxide elimination measurement--a two-center study. Clin Nutr 2015; 34: 151-155

**Changes in the text:** see page 6, line 148, reference 14

3. Describe the conditions of blood sampling taken by the participants of the study.

**Reply:** We would like to thank the reviewer for this comment. We have added an description in our methods: *“After overnight fasting, blood samples of participants were obtained and centrifuged at 4.0°C for 10 min at 1000 rpm and subsequently analyzed”*.

**Changes in the text:** page 5, line 121-122

4. Overall the findings discussed in this manuscript such as the reduction of muscle mass with ageing or the lower insulin levels with increased muscle mass lack novelty since these effects have been reported in numerous previous classical studies.

**Reply:** We would like to thank the reviewer for this comment. We believe that being able to reproduce other people’s findings is a positive thing, and beneficial to the field.

**Although we have included replicated data, we have also produced some novel data – that is, the findings relating FORs and exercise intensity in the context of ageing-related muscle loss. Our results are novel in that it is the first time this kind of research has been done on a Chinese cohort.**

5. The authors did not place any information of the important physiological factors, such as food intake, dietary records and physical activity etc.

**Reply: We would like to thank the reviewer for this comment. We understand this limitation and it is noted in our discussion section.**

**Changes in the text: page 14, line 324-331**

6. The authors claim that they studied 224 healthy active men. The large life span of the participants in this study ranging from 23-92 years of age does not warrant comparable levels of health and activity. More details and a stronger rationale is needed here.

**Reply: Thank you for identifying this limitation. The study was designed specifically to encompass a large age range as we were researching the effect of age on the data collected. You raise a good point regarding the difficulty in direct comparisons, given the likely differing levels of health and activity. We have thus expressed this limitation in our discussion section.**

**Changes in the text: page 14, line 324-331**

7. The authors show the correlations they want to show. The biological markers have been largely ignored or are not shown.

**Reply: We would like to thank the reviewer for this comment. All biological markers are shown in Table 1 of the manuscript. We have added the non-statistically significant markers in the results section to reflect a more holistic analysis.**

**Changes in the text: page 8, line 187-191**