Author’s response to reviews

Title: Intermittent whole-body vibration attenuates a reduction in the number of the capillaries in unloaded rat skeletal muscle

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Version: 3
Date: 30 August 2014

Author’s response to reviews: see over
Title: Intermittent whole-body vibration attenuates a reduction in the number of the capillaries in unloaded rat skeletal muscle
(Old title: Preventive effects of intermittent whole-body vibration and weight bearing on capillary number reduction and muscle atrophy in unloaded rat skeletal muscle)

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Version: 2
Date: 30 August 2104
Author’s response to reviews: see over
Reviewer: Mary Barbe
Reviewer’s report:

Major Compulsory Revisions

1. The authors should consider a new title that indicates that whole-body vibration and weight bearing only partially attenuate changes in unloaded muscles.

   We agree with the reviewer’s suggestion and the title was changed as follows:
   “Intermittent whole-body vibration attenuates a reduction in the number of the capillaries in unloaded rat skeletal muscle”

2. While the topic of the study is of great importance (the use of interventions to prevent atrophy and reduced numbers of capillaries in muscles occurring with disuse), the findings are overstated in several places. This is primarily because the weight bearing and intermittent vibration interventions used either had no significant effect, compared to controls, or only partially attenuated the effects of hind-limb suspension for most of the variables assayed. The author’s findings would be greater use to other researchers if they could please scale back their conclusions to state that VIB only partially attenuated muscle atrophy and the decrease in capillary numbers. This should occur in several places including the abstract (page 3, lines 57-58, in which it is indicated that VIB “was effective”, although it only partially attenuated HS induced changes), and throughout the discussion.

   I agree with the reviewer’s suggestions and rewrote to tone down our description as “Our results suggest that weight bearing with or without vibration is effective for disuse-derived disturbance by preventing muscle atrophy, and VIB exercise has an additional benefit of maintaining microcirculation of skeletal muscle.” (P3, L57-60, colored)

For example, on page 15, lines 281-282 where the authors state that “attenuation of all of these pro-angiogenic factors were considered to be involved in capillary reduction in HS”. Since only VEGF-A and CD36 changed significantly compared to controls, only these factors should only be addressed here, please.

   Description was changed according to the referee’s comments as below.
   “In the current study, it was demonstrated that angiogenic-relatated factors, such as VEGF-A and CD36 was considered to be involved in capillary reduction in HS group.” (P15, L286-288, colored)
Also, please 17, lines 319 through 322. The findings that were not statistically significant should be considered as such. The authors should scale back on these statements. In these sites, and others in the discussion and final conclusion, the authors need to be careful to not overstate their findings. More clearly indicating that the interventions were only partially successful (or not at all in some instances) in attenuating HS induced changes, will aid other researchers in their path to find either other inventions or alterations in these interventions that will rescue the disuse-losses better.

We agree with the reviewer’s suggestion and rewrote to tone down our description as “The former mechanism may be indicated by the graded decrease in the expression of CD36 mRNA among the HS groups (Fig. 4), although the difference was not statistically significant.” (P18, L329-331, colored)

We added the word “partially” to the sentence. (P3, L55, P17, L325, P21, L392 and 395, colored)

3. The aims of the study are not stated completely. Page 5 states: “In this study, the effects of VIB were examining using histological techniques on disuse-induced atrophied muscle to show changes in muscle vascularization in the rat soleus muscles”. Please restate to include the use of weight bearing and the use of mRNA methods, in addition to the histological techniques.

We agree with the reviewer’s suggestion and rewrote the sentences as

“In this study, whether whole-body vibration with weight bearing are more effective in preventing disuse muscle atrophy than weight bearing alone was examined in the aspects of muscle volume and muscular vascularization using real-time PCR and histological techniques on disuse-induced atrophied muscle. (P5, L89-92, colored)

4. Can the authors please speculate in the discussion if variations of their weight bearing and intermittent whole-body vibration could possible lead to better outcomes?

To address these questions, we added the following sentences:

“A previous study indicated that intermittent weight bearing intervention on unloaded hindlimb prevents muscle atrophy in a time-dependent manner [23]. Therefore, prolongation of both VIB and WB intervention time (> 20 min/day) would lead to better outcome. Further studies are necessary to confirm the hypothesis.” (P20, L371-374, colored)
Also, in the studies from other labs that had different results from this study, were the intervention methods different (time of weight bearing per day, cycles of vibration, etc).

To address these questions, we added the following sentences:

In Methods

“Total of 20 min of weight bearing time per day may be insufficient according to the previous report [23], but we chose this length to test the synergic effects of concomitant vibration for 16 min.” (P7, L127-129, colored)

In Discussion

“In contrast to the current study, a previous study reported that 6 weeks of daily intermittent whole-body vibration induced a reduction in capillary number in mouse soleus muscle [19]. To explain this discrepancy, we propose two possibilities. First, alteration of endothelial cell function by hindlimb suspension [44] might be involved in difference in the sensitivity of lower limb vascularization to whole-body vibration. Second, differences of stimulus condition (e.g., vibration frequency and amplitude) might affect the outcomes of vascularization, because changes in both vibration frequency and amplitude affect human leg blood flow velocity [43]. Therefore, optimal stimulus condition should be investigated in further studies.” (P18, L346, P19, L347-354, colored)

5. Is it common to randomly assay muscles? I was under the impression that zones of muscles should be sampled in a consistent manner since some regions may alter while other may not.

We believe it is common to randomly choose areas to assay to estimate C/F as long as specimens were always cut at the mid-belly portion (Methods/Tissue preparation P8, L140-143, colored). Also in a previous study, where capillary numbers were counted in areas randomly chosen from cross-section of rat soleus muscle in order to estimate of C/F (Roudier et al., 2010, J Physiol). In addition, we do not find regional difference by microscopic observation. Thus, we believed that our method was suitable for the histometrical assessment and the obtained data are acceptable.

6. The statistical section should indicate which statistical method was used for which assay/outcome.

We agree with the reviewer’s suggestion. In order to clarify this point, statistical section has changed as “For body weight, relative muscle weight, and gene
expression of VEGF-R2, one-way analysis of variance (ANOVA) and the Tukey’s post-hoc test were applied. For C/F ratio and gene expressions of VEGF-A, TGF-β1, and TSP-1 which assumption of homoscedasticity was not met for data, ANOVA and the Dunnett’s T3 test were alternatively applied. For CSA and gene expression of CD36, the Kruskal–Wallis test was applied, followed by a Mann–Whitney U test with Bonferroni adjustment.” (P12, L218-223, colored)

Minor Essential Revisions

1. The authors need to put labels or clearer labels on the y-axis of figures 2-4.
   As reviewer’s indicated, labels position was corrected on the y-axis of figures 2-4 (colored).

2. C/F needs to be defined in the methods. It can be deduced from the abstract, and is defined in the abbreviations list, but could benefit from a definition here on first use too, please, similar to the other abbreviations.
   Please see the revised manuscript. The first use of C/F is in Background section (P4 L70, colored), and C/F was defined in there. In addition, we redefined in the Methods section. (P9, L172, colored)
Reviewer: Naoto Fujita
Reviewer’s report:

Minor essential revisions

#1 The authors state “Importantly, VIB concomitantly prevented a reduction in the number of capillaries and muscle atrophy during unloading. Our results suggest that VIB exercise is effective for disuse-derived disturbance by preventing muscle atrophy and maintaining microcirculation of skeletal muscle” as conclusions in abstract section. However, there were no significant differences in muscle weight and muscle fiber area between the HS + WB and HS + VIB groups. The statement implying vibration is effective on muscle atrophy should be deleted to avoid misunderstanding.

    We agree with the reviewer’s suggestion. In order to clarify this point, we changed the description as below.

    “Importantly, only VIB could partially attenuate a reduction in the number of capillaries during unloading. Our results suggest that weight bearing with or without vibration is effective for disuse-derived disturbance by preventing muscle atrophy, and VIB exercise has an additional benefit of maintaining microcirculation of skeletal muscle.” (P3, L57-60, colored)

#2 The introduction does not clearly state the hypothesis and objective in this study. The readers want to know “what is this study interesting?” Please emphasize your research question in introduction section.

    We removed some descriptions in the introduction section of the original manuscript, and made it straightforward for the clarity with some additional explanations. (P5, L84-88, and P5, L92-94, colored)

#3 The authors should mention the reason why vibration performed 20 min in a day.

    The reason for the use of 20 min length of weight bearing with vibration was included in the method section (P7, L127-129, colored), as “Total of 20 min of weight bearing time per day may be insufficient according to the previous report [23], but we chose this length to test the synergic effects of concomitant vibration for 16 min.”

    Additionally, what is the reason for vibration frequency of 55Hz? If the reason is shown in the manuscript, the statement gives readers possible application.
In regard to vibration frequency, the explanation was added in the method section: “Vibration frequency was set at 55 Hz as a maximal value of the whole-body vibration platform, because lower-limb muscle activities increased depending on the increase of vibration frequency [21, 22]” (P7, L121-123, colored)

#4 In Table 1, although there is no significant different in body weight between the CONT and HS groups, the values are significantly lower in the HS + WB and HS + VIB groups than the CONT group. Were weight bearing and vibration high intensity exercise? Please provide details regarding how the exercise intensity was determined.

Please see answers in #3 for the determination of exercise duration and intensity. The reviewer pointed out accurately. However, in regard to increasing rates of body weight, there were no differences among the HS (+7%), HS + WB (+1%), and HS + VIB (+4%) groups (P = 0.13). Thus, we assume that weight bearing and whole-body vibration are low intensity exercise. Differences of body weight at the end of experimental period might mainly reflect the differences at onset of experiment.

#5 Generally, the number of capillary depends on muscle fiber size and the type distribution. Therefore, if the treatment could prevent capillary regression by unloading, the muscle atrophy must be also attenuated by the treatment.

However, the vibration treatment in this study was effective only in capillary. Please provide the reason the effect was just in capillary. It is readily understood that reduction of unloading time in a day can prevent disturbance with disuse. The authors should mention what property of vibration is effective on prevention of capillary regression.

Both muscle atrophy and reduction in number of capillaries by hindlimb suspension were partially prevented by whole-body vibration with weight bearing (Control vs HS + VIB group). Thus, from the present study, it is difficult to isolate pure effects of vibration on disused muscles, but additional benefits on vascular developments or maintenance were demonstrated in HS + VIB group. Changes in capillary numbers are not necessarily parallel to changes in muscle activity. For example, increase in blood flow by drug administration increases the capillary to muscle fiber ratio in skeletal muscle (Bongrazio et al., 2006, Int J Immunopathol Pharmacol). Therefore, we speculate that the same mechanism (increase in blood flow velocity due to whole-body vibration) worked against capillary regression in this study as in the case of drug administration. We added references in the discussion section that support human whole-body vibration increase lower-limb blood flow: “In human,
whole-body vibration causes increase in lower-limb blood flow velocity which augments shear stress.” (P18, L352-353, colored)

#6 In Fig. 4, some of symbols are not centering.
Symbols were corrected. (Fig 4, colored)

Discretionary revisions:

#1 The authors focus on preventive effect of vibration in this study. However, other control group is essential to strictly proving preventive effect. If possible, I suggest you add two experimental groups that received vibration treatment without unloading and received the treatment during unloading without weight bearing.

Further exploring pure effects of vibration on disused muscle must be attractive. However, this study was designed to test the effects of whole-body vibration as in a clinical setting which needs weight bearing. Thus, we hope this manuscript in the present form is still useful for readers who search better remedies for disused muscles.

#2 There are some abbreviations in this manuscript. For example, I could not judge immediately whether HS means the HS group or just suspension procedure in some parts. The VIB and WB were also the same. Unfamiliar abbreviations should be used only as experimental group name.

The relevant sentences were corrected.