Author's response to reviews

Title: Time-course of Exercise and its Association with 12-Month Bone Changes

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Author's response to reviews: see over
Dear Dr. Graham,

Thank you for your e-mail message on March 25th on manuscript 8442013352333698 entitled “Time-course of exercise and bone changes in a 12-month exercise trial”.

Please find enclosed our revision, in which we have considered the comments given by the reviewers. A detailed list of actions taken during the revision is also provided.

We hope that the revised version of the manuscript is found suitable for publication in BMC Musculoskeletal Disorders.

Kind regards,
Riikka Heikkinen
Corresponding author
RE: BMC Musculoskeletal Disorders manuscript 8442013352333698

Referee 1 (Katherine Brooke-Wavell):

We appreciate the comments given by the reviewer. We agree that the individuals having higher accelerations in the early phases of the intervention are also likely to have higher accelerations subsequently. We have now adjusted our statistical analyses for the accelerations at the later phases of the intervention.

A detailed list of actions taken during the revision:

Major revisions:

1. Please use an analysis that will determine whether associations between daily impacts in months 0-3 or 6 and bone outcomes were independent of daily impacts in later months.

   We have reanalyzed the associations by using multivariate models adjusting for daily impacts in later months.

2. To determine time course of bone change (as implied by the title) or the outcome of exercise intensity (as stated in the aim) requires measurements of bone changes at intermediate time points.

   Unfortunately we had bone outcome data only at baseline and 12 months. We agree that the study would have been stronger if we had the intermediate data. The aim and title have been now modified.

3. Pearson's product moment correlation coefficients are reliant on the distribution of data and measurement range and are highly influenced by outliers. Please confirm that data were normally distributed and preferably provide a scatter plot of the key associations to demonstrate whether outliers are present that might inflate correlations.

   We have now included an example scatter plot to demonstrate the distribution of the data. Please see new Fig. 1. In addition to correlation analysis, multivariate models have now been added.

4. When conclusions are being drawn from comparing correlation coefficients, please indicate whether these differ significantly.

   The statistical analysis has now been re-performed and the text has been modified accordingly.

5. Please describe how many days of activity data were available at each stage of the study and what procedure was used for missing data.
The minimum requirement was two weeks of continuous measurements per period. Subjects with less accelerometer data were excluded from the analysis. We checked missing days from the accelerometer data and used average daily numbers of impacts in which we took into account accelerometer compliance.
We appreciate the comments given by the reviewer.

A detailed list of actions taken during the revision:

Statistics: After statistical consultation, the statistical analyses were reperformed using multivariate analysis. Results were adjusted for daily impacts in later months and other possible confounding factors, such as baseline differences.
RE: BMC Musculoskeletal Disorders manuscript 8442013352333698
Time-course of Exercise and Bone Changes in a 12-Month Exercise Trial

Referee 5 (Jose A L Calbet):
We appreciate the comments given by the reviewer.

A detailed list of actions taken during the revision:

Major comments:

The referee expresses his wish of having the effects of exercise intervention (BMD and QCT data) reported as well as control group.

To avoid duplication of results, BMD and QCT results were not included here, since they have been reported earlier in several our papers (Vainionpää et al. Osteoporos Int. 2005, Osteoporos Int. 2006, Bone 2007) and we have referred to these papers through the manuscript.

The setting (originally a randomized controlled population-based) and protocol of the trial have also been thoroughly presented in these previous papers. The novel aspect of this paper is that it examines whether impact loads from baseline to 3 or 6 months are associated with bone changes after 12 months. Thus, we think that including the control group is not relevant for this analysis.

Some of the changes in bone mass may just be seasonal.
The bone mass evaluation was performed in baseline and after 12 months (±2 weeks), which minimizes the effect of any seasonal variation.

Carry over effect
Unfortunately we had bone outcome data only at baseline and 12 months. We agree that the study would have been stronger if we had the intermediate bone data. We used intervals 0-3, 0-6, and 0-12 months for exercise (cumulative), because exercise effects are cumulative as well. We have now reanalyzed the associations by using multivariate models adjusting for daily impacts in later months.

Methods:
1) Inclusion and exclusion criteria as well as drop outs have been published previously (Vainionpää et al. Osteoporos Int 2005, 2006, Heikkinen et al. J Biomech 2007).
   Inclusion criteria of the original intervention (a random population-based sample of women) were age 35–40 yr, residing in the city of Oulu, Finland in March 2002. The exclusion criteria were cardiovascular, musculoskeletal, respiratory, or other chronic diseases that might limit training and testing; diseases or medication affecting the bone;
pregnancy and breast-feeding; and regular current or previous participation in impact-type exercises and long-distance running more than three times a week. We have now described that subjects of this study were healthy and were not largely involved in high-impact sports. The exercise group consisted of 60 subjects, of which 39 completed the study. The reasons for withdrawal were medical problems unrelated to the intervention program (n=3), pregnancy (n=4), moving from the study area (n=2), change of vocation or schedule (n=6), incompliance with long-term use of body movement monitor (n=3), and other reasons not known (n=3) (Vainionpää et al., 2006). Four subjects missed physical activity data, and thus n=35 in this study.

2) Previous exercise history: The original intervention was population-based, and one exclusion criterion was that the subjects were currently not participating in impact-type exercises or long-distance running more than three times a week. A modified Paffenbarger questionnaire was used to evaluate lifetime physical activity at the age of 15, 20 and 30 and at baseline. There were no athletes at national or international level within our subjects. At the age of 15, four subjects reported heavy exercise >5 days per week, at 20 years n=3, and at 30 years n=3. At baseline (age of 35–40), no subject reported heavy exercise 5 or more times per week.

3) Menstrual status: There were no oligomenorrheic or amenorrheic women in our data. Seven women reported occasional minor irregularities in menstruation.

4) Oral contraceptives were used by 39.4% of the subjects (reported in Vainionpää et al. 2006). This has now been added in Subjects.

5) Calcium intake: Mean 1,101.1 (SD 532.7) mg per day reported in Vainionpää et al. 2006. This has now been added in Subjects.

6) Trochanteric region was defined according to DXA manufacturer’s (Hologic) standard protocol.

7) Prevalence of smoking was assessed with a questionnaire and was 21.2% in this group (Vainionpää et al. 2006). This has now been added in Subjects.

We have now included more descriptions of the subjects in the methods. However, we consider that the details have been published previously as mentioned above.

Exercise was group-based supervised step aerobic with music. The exercise protocol has been described in detail in our previous papers (Vainionpää et al. Osteoporos Int 2005, 2006, Bone 2007). Anyway, some more details are now given. The number of jumps per session may not be analyzed in our data. Forty minutes of each session was devoted for high-impact training. The
number of training sessions offered was kept constant (three times per week) during the trial. Running refers here to running steps on the spot during the supervised step aerobic routine. Thus, we can not give km values per week. Instead, we report the number of impacts measured. We have now stated this more clearly under Exercise program.

The accelerometer used in this study is available from Newtest Ltd., Oulu, Finland (Pat. US7198607 and US 2008312560). The threshold for acceleration measurements (minimum acceleration measured) was 0.3g. This has now been added to Methods/Physical activity measurements. The interval of the levels in the acceleration histogram was not constant: it was 0.2g, at low g levels (in which the numbers of impacts fall very dramatically when g level gets higher) and increased up to 0.6g at the highest acceleration levels (where the daily numbers are very low and there is no dramatic change between levels). Please see an example histogram of daily impacts (of an active athlete, from a separate study) below.

The five levels to describe exercise intensity were combinations (summation) of these 32 levels and thresholds were chosen to describe typical activities (0.3–1.0g e.g., walking, 1.1–2.4g e.g., stepping, 2.5–3.8g e.g., jogging, 3.9–5.3g e.g., running and jumping, and 5.4–9.2g e.g., jumping and drop-jumping) based on our pilot study. Please see Fig. 2 in Vainionpää et al. 2006, Osteoporos Int paper. This procedure has now been more carefully explained in Methods.

Ground reaction force was measured with a force plate (Kistler 9287A with a Kistler 9865C charge amplifier). $R$ represents here Pearson’s correlation coefficient. We referred to peak ground reaction force compared with peak acceleration value and area under the peak curve. We have refined this in Methods, as suggested.

The correlation coefficients ($R$) were not compared statistically with each other. We have now reanalyzed the associations by using multivariate models, adjusting for daily impacts in later
months and other potential confounding factors. Statistical method and results have been rewritten.

**Discussion**

In the present study, we did not discuss the magnitude of bone changes because these issues have been discussed earlier in Vainionpää et al. 2005, 2006, Osteoporos Int papers and 2007 Bone paper and we think are out of the scope of this paper.

*This study can not establish which impacts are more osteogenic based only on the R values.*

The discussion and conclusions have been modified according to this and other comments.

**Conclusion**

We have modified the conclusions as suggested.

**References**

We thank for this reference of a very recent study and have commented that in discussion.

**Minor comments:**

P5, L7: Here we consider running as running steps during the step aerobic routine. Walking has been removed from high impact exercise in order not to involve it in high impact activities. We agree that strain is dependent on running velocity; however it has been shown that slow jogging (bouncing) creates greater GRFs than fast running or walking at the same speed (Keller et al. Clin Biomech 1996). Ground reaction force recordings cannot be directly compared with acceleration values measured at the hip. We have modified this paragraph (please see above).

P11, first Paragraph: As suggested, we have now added a reference here.

P12, L3. Modified as suggested.

P12, L4. We have formatted the reference by Cullen et al. according to suggestion.

*Number of jumps per week* Please see reply above

*Number of impacts per week* We agree that number of impacts graph per week would be good. However, we recorded approximately a total of 150 million acceleration peaks during the
intervention study, and it would need a manual analysis file-by-file subject-by-subject to receive this graph.

*Progression*

We reported cumulative values of impacts (0-3, 0-6, 0-12 months’ averages) which overlap, and thus progression is not clearly visible in these averages.

*Why N=34 in Figure 1 and not 35?*

In Fig.1. (Fig.2 in revised version), N=34 because we had DXA data was missing from one women. This has now been added to figure caption. Figure caption has been modified according to comments by other reviewers.
Referee 2 (Olivier Bruyere):

We appreciate the comments given by the reviewer.

A detailed list of actions taken during the revision:

Major revisions:

- **Interpretation of the results**

  Unfortunately we had bone outcome data only at baseline and 12 months. We agree that the study would have been stronger if we had the intermediate data. The aim and title and conclusions have been modified. We also reanalyzed the associations by using multivariate models adjusting for daily impacts in later months, as suggested by the reviewers. Please see also our responses to reviewers 1 and 3.

- **Statistical analysis**

  We reanalyzed the associations by using multivariate models, adjusting for potential confounding factors.

- **Abstract should include data on the relationship between impact activity and bone geometry at the proximal and distal tibia**

  As suggested, we have now included data on the relationship between impact activity and bone geometry at the proximal and distal tibia in the abstract.

- **The authors should be slightly more balanced regarding the first two sentences of their introduction.**

  We have now removed the first sentence to balance the introduction, as suggested.

- **Exercise program: adjustment for compliance.**

  Compliance has now been used as a covariate in multivariate analysis, as recommended.

- **CV of DXA and QCT measurements**

  The CV values were obtained in a clinical setting using well-qualified personnel. We have now added CV also for tibial QCT.
- *It could be interesting to show the change in BMD and the change in bone geometry (in %) in function of the average daily numbers of impacts*

The data have been previously presented (Vainionpää et al. Ost Int 2006, Bone 2007). An example scatter plot has been included (Fig. 1 in revised version).

- *A table with baseline characteristics*

The complete table of baseline characteristics has been reported earlier (Vainionpää et al. 2006, 2007). We have now included the main characteristics of the subjects in the text.

- *A table with change in BMD and bone geometry*

We also agree that a table with BMD and bone geometry changes could be interesting, but to avoid duplication of results they were not included here, since they have been reported earlier (Vainionpää et al. Osteopor Int 2005, Bone 2007, Heikkinen et al. J Biomech 2007).

- *Correlation between impact activity and proximal or distal tibia geometry*

This is shown below. Since there were no significant correlations, the table was not included in the manuscript. We have now discussed the lack of associations in tibia, as suggested.

<table>
<thead>
<tr>
<th>Average daily numbers of impacts at different acceleration levels</th>
<th>0–3 months</th>
<th>0–6 months</th>
<th>0–12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3–1.0g</td>
<td>1.1–2.4g</td>
<td>2.5–3.9g</td>
<td>3.9–5.4g</td>
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<td>1.1–2.4g</td>
<td>2.5–3.9g</td>
<td>3.9–5.4g</td>
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<td>2.5–3.9g</td>
<td>3.9–5.4g</td>
<td>5.4–9.2g</td>
<td>0.3–1.0g</td>
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<td>3.9–5.4g</td>
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<td>5.4–9.2g</td>
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<td>2.5–3.9g</td>
<td>3.9–5.4g</td>
<td>5.4–9.2g</td>
</tr>
</tbody>
</table>

**Proximal tibia**

- Bone circumference: 0.13 0.19 0.19 0.09 0.08 0.07 0.14 0.24 0.20 0.07 0.10 0.08 0.20 0.18 0.10
- Cortical CSA: 0.09 0.01 0.05 -0.02 -0.05 0.11 0.08 0.01 0.01 0.01 0.19 0.09 -0.01 -0.02 -0.02
- Cortical attenuation: -0.12 -0.01 0.03 0.08 0.04 -0.07 0.08 -0.05 -0.01 0.08 -0.04 0.13 -0.03 -0.01 0.04
- Max cortical CSMI: 0.16 0.22 0.20 0.10 0.13 0.11 0.20 0.33 0.25 0.10 0.13 0.13 0.30 0.24 0.12
- Cortical thickness: -0.22 -0.25 -0.19 -0.10 -0.08 -0.15 -0.16 -0.28 -0.20 -0.03 -0.13 -0.09 -0.25 -0.20 -0.08

**Distal tibia**

- Trabecular attenuation: -0.15 0.14 0.15 0.19 0.15 -0.05 0.18 0.04 0.11 0.17 -0.03 0.21 0.03 0.08 0.14
- Figure 1 could be replaced by an exhaustive table

Fig. 1 (= Fig. 2 in revised version) has now been modified according to recommendations by reviewer 3.
RE: BMC Musculoskeletal Disorders manuscript 8442013352333698
Time-course of Exercise and Bone Changes in a 12-Month Exercise Trial

Referee 3 (Robin Daly):
We appreciate the comments given by the reviewer. A detailed list of actions taken during the revision:

Major revisions:
*My main concerns relate to the accelerometer data and how it was presented and analysed.*
We did not control accelerometer use each day for example in a form of a diary. This would have been exhaustive for the subjects, since they were asked to wear the accelerometer every day and fill in several additional questionnaires and forms during the study. The data were downloaded after exercise classes, approximately every second week. Our analysis script analyzed each file for how many days the device was worn and the output was the average daily numbers according to this. The minimum requirement of accelerometer data was two weeks of continuous measurements per period. Subjects with less accelerometer data were excluded from the analysis. We agree that the variation in the length of each measurement period may have caused some variation in the average daily numbers. However, we do not think this causes too much error because we used long-term averages (3, 6, 12 months) of exercise in the final analysis.

*Compliance to exercise program*
We agree that compliance was not too high. First, the original intervention setting was population-based, which may have affected compliance. We recruited subjects from the whole age cohort residing in a specified area. Furthermore, we excluded the women already involved in high-impact exercise, thus we certainly also excluded the highly motivated subjects. The women of this age group have numerous family responsibilities and career obligations which also lower compliance. Compliance has now been used as a covariate in multivariate analysis, as recommended.

*Is there a reason why total hip data was not reported?*
Total hip data was not reported in this study, because only variables significantly associated with exercise data in our previous study (Vainionpää et al. 2006) were included.

*P7. Cortical bone separation from trabecular bone*
Threshold of 450 HU was used to separate cortical and trabecular bone.
P8. *QCT calibration phantom (Cortical attenuation and vBMD)*

The explanation for CSMI has been reworded. Due to the principle of CT technology, HU and vBMD carry the same information (see e.g. Lagravère MO et al. 2006 Dentomaxillofacial Radiology), even though the uncalibrated data can not give true bone density values. We agree that our results concerning cortical attenuation are somewhat conflicting with the previous data on vBMD. The cortical attenuation results of premenopausal women are now discussed.

P9. The first paragraph of the discussion has been rewritten according to the comment.

P11. As suggested, we have specified “cortical” in geometrical adaptation.

**Minor revisions:**

*Please clarify what you mean by 50% of the estimated bone length from the distal endplate of the femur. Was femoral bone length physically measured or just estimated?*

The length of the femur was estimated by measuring it manually using a ruler.

*Table 1. The results for the 0-12 month accelerometer data in this paper are slightly different than to those reported for the exercise group in the authors Osteop Int publication. Is there a reason for this difference?*

The results for 0-12 months are different, because in Osteoporos Int 2006 paper we included all subjects, also non-exercisers. In the QCT paper (Vainionpää et al. Bone 2007) we reported exercise group values separately, but presented relative daily numbers of impacts (relative to the mean of the controls). In the present study we used absolute daily numbers of impacts, since control group was not included in the analysis. This explains the slightly different results.

*Figure 1. modifications*

The figure (Fig. 2 in revised version) has been modified as suggested. The symbols for significant correlations have been included as suggested and the scales of the axes have also been changed.