Author's response to reviews

**Title:** A 12-week after-school physical activity programme improves endothelial cell function in overweight and obese children: a randomised controlled study

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**Author's response to reviews:** see over
BMC Pediatrics

Section Editors

2 July 2012

Re: Manuscript 1931930337675637.R1 A 12-week after-school physical activity improves endothelial cell function in overweight and obese children: a randomised controlled study

Dear Section Editors,

Many thanks for your letter dated 18 June 2012 regarding our paper. Once again, we appreciate the comments of the reviewers and thank you for giving us the opportunity to revise and resubmit our paper.

We have modified our paper on the basis of the reviewers’ comments. Our point-by-point response to each of the reviewers’ comments is included.

Should you require clarification regarding any aspect of the paper please do not hesitate to contact us.

We look forward to hearing from you in due course.

Yours sincerely,

Sang-Kab Park

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A 12-week after-school physical activity improves endothelial cell function in overweight and obese children: a randomised controlled study

General comments

The authors wish to thank both reviewers for reading our revised manuscript and once again providing constructive comments. Our responses, and the necessary changes (i.e. to the Reviewer 1, highlighted in yellow; to the Reviewer 2, highlighted in green) are included within the revised document.

Responses to Reviewer 1

Major Compulsory Revisions (4):

Query 1: In the methods section under the sub-heading "participants", the authors state that the children were assigned randomly to the control or exercise group; however, in response to reviewer 2, the authors state that they made some efforts to match the subjects for sex. This should be included in the methods section.

Response 1: Thank you very much for your comments. We have added a sentence to clarify this point including in the methods section (Page 6, Line 24).

Query 2: In the Methods section under the sub-heading "measurement of endothelial progenitor cells", the 8th sentence states that the endothelial progenitor cells were defined as "triple positive" events. While the authors did look at 3 categories of cells (CD34+, CD 133+, and CD34/CD133+), a third marker was not used that would make the cells triple positive. Only double positive cells were classified as endothelial progenitor cells.

Response 2: We do apologise that it is not triple positive – it was our mistake. We have modified the word “triple” to the word “double positive” throughout our revised manuscript (Page 10, Line 3; Page 25 Line 6).

Query 3: In the Methods section under the subheading "other laboratory assays", it is still unclear exactly when the second round of testing took place. While it does state that the testing was done after a period of 48 hours without exercise, it does not give the
average duration of time since the last exercise session. Were all the tests done 48 hours after the last exercise session?

Response 3: Thank you very much for your comments. We confirm that all blood samples were collected 48 hour after the last exercise session. Therefore, we have rewritten several sentences in the Methods section to clarify our procedure (Page 10, Lines 9-12).

Query 4: In figure 1, a tight cloud of double positive cells are apparent with rather strong signal intensity; however, a group of cells is also included in the current gating scheme that is much lower in fluorescent intensity. It would be helpful to also see the isotype control to help with the justification of including these lower intensity fluorescent cells.

Response 4: Thank you very much for your comments. We do apologise that it was our mistake - we did not perform the isotype control analysis in present study. However, a tight cloud of double positive cells are apparent with rather strong signal intensity. Therefore, please consider to accept our original result.

Minor essential revisions (4):

Query 5: In the first sentence in the background section please replace the word "clogged" with plaque or significant atherosclerosis.

Response 5: Thank you so much for your comments. We have replaced the words “clogged” with “plaque”. (See Page 5, Line 2 of the revised manuscript).

Query 6: In the same paragraph, please add a comma after the word “45-year-old”.

Response 6: We have added a “,“ (i.e. comma) after the word 45-year-old. (See Page 5, Line 6 of the revised manuscript).

Query 7: In the results section, the paragraph describing changes in blood parameters, please remove the work trends in regards to HDL and adiponectin. Considering the p-values for these 2 blood parameters, chance could also explain the relationship 38% and 29% of the time respectively.
Response 7: Thank you so much for your comments. I agree with your comments, we have deleted the following sentence from manuscript: “but there were trends toward increases in the exercise group (from $1.1 \pm 0.1$ to $1.4 \pm 0.1$ mmol/L; from $14380 \pm 1290$ to $15950 \pm 1731$ ng/mL, $p = 0.379$; $p = 0.293$, respectively)”.

Query 8: In the results section, the paragraph containing the endothelial progenitor cell data, please add (group x time) before the word interaction in the first sentence.

Response 8: Thank you so much for your comments. We have added the “(group x time)” before the word “interaction” in the first sentence of results section. (See Page 12, Line 25 of the revised manuscript).

Responses to Reviewer 2

Specific Comments (9):

Query 1: The authors wrote: “Furthermore, the study of Le and colleagues found that the carotid artery intima-media thicknesses of obese children were more typical of those of a 45-year-old and these children had high levels of LDL and triacylglycerol and low levels of HDL [1].” The authors should clarify whether they mean that the obese children had cIMT values that were similar to normal healthy adults, or adults with obesity or related metabolic disorders. Please revise this sentence to be more specific.

Response 1: Thank you so much for your comments. Based on your comments, we have modified these sentences in the Background section (Page 5, Lines 5-6).

Query 2: The authors wrote: “In addition, our power calculation assumed at least a 70% increase in CD34, CD133, CD34/CD133 and CIMT.” Did the authors really expect to observe a 70% increase in EPCs and cIMT? Those seem like pretty large changes to predict. More importantly, please either explain why you expected cIMT to increase since this was the opposite effect of what was observed and what has been shown in the literature for people who exercise and/or lose weight. Perhaps this was not written as clearly as intended and should be revised.
Response 2: Thank you so much for your comments. We do apologise that the word “CIMT” should not be here – it was our mistake. We have deleted the word “CIMT” from our revised manuscript.

Query 3: The authors wrote: “Participants were advised to avoid physical activity for 48 hours and to fast for at least 10 hours prior to sample collection.” This new sentence was added in response to a comment made in the prior review, which is good. However, it would be even better to clarify whether this timing applied to both the beginning and end of the study and for the all of the tests besides blood collection. That is, once the child had finished the last supervised exercise session in week 12, how much time passed until each of the outcome tests was performed: cIMT, blood for EPC, and the body composition and treadmill tests? This type of detail is important for exercise intervention studies because it allows the reader to decide whether the changes observed are due to adaptations to training, rather than the residual effect of the last exercise session.

Response 3: Thank you so much for your comments. We apologise for not clearly describing the methods section. As Reviewer 1 also raised this issue, we confirm that all the measurements were collected 48 hours after the last exercise session. Therefore we have added a sentence in the Methods section to incorporate this suggestion from the reviewer and have rewritten several sentences to clarify our procedure (Page 7, Lines 20-21; Page 10, Lines 9-12).

Query 4: In response to a prior comment from this reviewer the authors stated that the two study groups were matched for the distribution of boys and girls. This clarifying reply appeared in their letter to the reviewers but the details were not added to the Methods. This information should be stated, along with any other stratification approaches that were used to balance the groups at the beginning.

Response 4: Thank you very much for your comments. We have added the details in the Methods section (Page 6, Line 24).

Query 5: Tables 1 and 2 have duplicate information on the outcome variables and could be combined into a single table. Add the first 3 lines in Table 1 (variables: sex, OW/Ob, age) to Table 2. Age and sex wouldn’t change during the study of course, but since BMI did change, it would be useful to list the number of OW/Ob at both the beginning and
the end of the study, and add a comment on whether any children changed BMI categories. For example, in the exercise group did any OW children move into the normal weight BMI as a result of weight loss? Or children in the obese range move into overweight. Another suggestion is to revise the title of Table 2 to be more descriptive and accurate of the results presented. The data shown are pre-post intervention results, rather than "change" values as stated, and there is more than body composition and VO2max as listed in the title. A broader, perhaps more general title as in Table 1 would be better.

Response 5: Thank you very much for your comment. We have modified the Table 1 as below.

Table 1. Body composition and maximal oxygen uptake measured at baseline and after 12 weeks

<table>
<thead>
<tr>
<th></th>
<th>Exercise Group (n = 15)</th>
<th>Control Group (n = 14)</th>
<th>p-value (Interaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.1 ± 0.1</td>
<td>12.2 ± 0.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Sex, boys/girls</td>
<td>7/8</td>
<td>7/7</td>
<td>N/A</td>
</tr>
<tr>
<td>Overweight/obese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7/8</td>
<td>6/8</td>
<td>N/A</td>
</tr>
<tr>
<td>12 weeks</td>
<td>10/5</td>
<td>5/9</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.46 ± 0.02</td>
<td>1.47 ± 0.02</td>
<td>0.905</td>
</tr>
<tr>
<td>12 weeks</td>
<td>1.48 ± 0.02</td>
<td>1.49 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>52.0 ± 1.8</td>
<td>52.3 ± 1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>12 weeks</td>
<td>51.0 ± 1.6</td>
<td>54.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>24.4 ± 0.4</td>
<td>24.3 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>12 weeks</td>
<td>23.2 ± 0.4</td>
<td>24.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>82.5 ± 1.7</td>
<td>76.3 ± 1.9</td>
<td>0.001</td>
</tr>
<tr>
<td>12 weeks</td>
<td>79.9 ± 1.9</td>
<td>78.9 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>108 ± 3</td>
<td>110 ± 2</td>
<td>0.470</td>
</tr>
<tr>
<td>12 weeks</td>
<td>111 ± 3</td>
<td>111 ± 2</td>
<td></td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>62 ± 1</td>
<td>66 ± 2</td>
<td>0.663</td>
</tr>
<tr>
<td>12 weeks</td>
<td>63 ± 2</td>
<td>66 ± 2</td>
<td></td>
</tr>
<tr>
<td>Maximal oxygen Uptake (ml/kg/min)</td>
<td>Baseline 34.1 ± 1.5</td>
<td>35.9 ± 1.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Uptake (ml/kg/min) 12 weeks</td>
<td>37.8 ± 1.6</td>
<td>33.2 ± 1.0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; N/A, not applicable.

Query 6: It would be a good idea to clarify whether the waist circumference and VO2max showed statistically significant changes within either group. It is unclear whether the significant interaction effect is due only to the opposite direction of changes
in the two study groups or whether the exercise group experienced an increase in VO2 and decline in waist size, independent of the control no-exercise group.

**Response 6:** Thank you very much for your suggestion. We apologise for not clearly describing the Results section. Therefore, we have added several sentences in the Results section. (Page 12, Lines 4-9).

**Query 7:** The authors wrote: “However, we failed to find the relationship between endothelial progenitor cells and carotid intima-media thickness \( (r = -0.118, p = 0.329) \) (Data not shown).” This is an important comment in the discussion because although the rationale for including cIMT measurements should be somewhat obvious, the authors did not explicitly state that their goal was to look for association between changes in cIMT and EPCs. This could have been more clearly stated in the introduction; without providing a rational or statement of purpose about cIMT, non-specialist readers may view the inclusion of cIMT as somewhat secondary. As a technical comment, the actual statistical results should be moved to the Results and interpretation of those results should be within the Discussion. Also, the finding that changes in cIMT and EPCs were not related is interesting but it would also be valuable for the authors to also comment in the Results about whether there was a correlation at baseline between these two variables (i.e., was the beginning value of cIMT correlated with EPC number?).

**Response 7:** Thank you very much for your comment. Based on your comments, we have added this point to the Background section (Page 5, Lines 16-20).

We have moved the correlation of CIMT and EPCs results to the Results section and interpretation of those results in the Discussion section (Page 13, Lines 12-15; Page 16, Lines 21-23).

**Query 8:** The authors wrote: “In addition, differences in diet might also be a confounding factor [41].” This comment would be improved if the authors expanded it to state that they did not provide specific dietary advice to the children, teachers or families (is this correct?) but that unmeasured changes in diet could have contributed to the weight loss in the exercise group. It is possible that because these children had been selected for the intervention that either their own eating behavior or the diet provided and the behavior of their parents or school staff was affected. The literature supports
that interventions with exercise-only (without structured nutritional approach for weight loss) typically result in little or no change in body weight or BMI in adults or children over 12 weeks (although body composition can and does change in some studies). Although the change in EPCs reported in the current study is most likely due to the exercise per se, the impact of body fat reduction secondary to dietary changes may also potentially contribute and this should be acknowledged.

**Response 8:** Thank you very much for your comment. I agree with your comment and this is important to acknowledge. Therefore, we have revised this point in the Limitation section as suggested (Page 17, Lines 17-21).

**Query 9:** Another limitation (or opportunity for future research) that should be stated in the final paragraph of the discussion is that the authors have not examined the separate effects of the aerobic versus resistive exercise. It is unclear whether these overweight children would have the same response if they only did one type of exercise.

**Response 9:** Thank you, we appreciate your comment. We have added this point to our discussion as limitations (Page 17, Lines 21-23).

We very much hope that you find these adjustments satisfactory and that the revised version will be acceptable for publication in *BMC Pediatrics*.

Sincerely yours,

Sang-Kab, Park