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

Authors:

Jong-Hwan Park (prof.parkjh@fuji.waseda.jp)
Masashi Miyasita (m.miyasita@aoni.waseda.jp)
Yoo-Chan Kwon (kwonyc@dau.ac.kr)
Hyun-Tae Park (tonypark@ncgg.go.jp)
Eun-Hee Kim (ehk1959@dau.ac.kr)
Jin-Kee Park (park7166@yahoo.co.kr)
Ki-Beam Park (welzel@dau.ac.kr)
Suk-Ran Yoon (sryoon@kribb.re.kr)
Jin-Woong Chung (jwchung@dau.ac.kr)
Yoshio Nakamura (nakamura@waseda.jp)
Sang-Kab Park (sgpark@dau.ac.kr)

Version: 3 Date: 26 May 2012

Author's response to reviews: see over
Re: Manuscript 1931930337675637 A 12-week after-school physical activity improves endothelial cell function in overweight and obese children: a randomised controlled study

Dear Section Editors,

Thank you for your letter dated 27 April 2012 regarding our manuscript (1931930337675637). We would like to thank the reviewers also for their constructive comments regarding our paper. We have revised the manuscript in view of these comments and attach here a revised draft of the paper for your consideration together with a point by point response to each of the issues raised by yourselves and the two reviewers. Thank you for giving us the opportunity to revise our manuscript. We look forward to hearing from you in due course.

Yours sincerely,

Sang-Kab Park

Sang-Kab Park, PhD.
Dong-A University, College of Sport Sciences
840 Hadan 2-dong, Saha-gu, Busan
604-714
South Korea
Phone & Fax: +82-51-200-7843
Email: sgpark@dau.ac.kr
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 the editor and two reviewers for reading our manuscript so thoroughly and providing such constructive feedback. Our understanding of area and the quality of our manuscript has certainly improved as a result of these 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. We have also listed the comments from each reviewer followed by our responses.

Responses to Reviewer 1

Major Compulsory Revisions (4):

Query 1: The manuscript could use some editing in regard to the English language. Incomplete sentences were common in the article for example the last sentence in the third paragraph of the background and in the discussion, the second paragraph, the sentence beginning with because. There are numerous other areas were potentially a word was left out or the wrong tense of the word was used. These all need to be addressed.

Response 1: Thank you very much for your comments. We apologise for the grammar mistakes in the initial version of this manuscript; we have endeavoured to correct them as described below.

The last sentence in the third paragraph of the Background and in the Discussion, the second paragraph has been modified as follows (Page 6, Lines 2-3; Page 13, Line 18 to Page 14, Line 6).

Therefore, a resistance training programme was included in a multidisciplinary weight management programme for overweight and obese children.

Endothelial progenitor cells promote angiogenesis and vascular repair, and enhance endothelial function [20]. Pluripotent haematopoietic stem cells also play a crucial role
in vascular repair and angiogenesis [17], and several recent studies report the potential health benefits of haematopoietic stem cell mobilization. Angiogenesis is a key factor in the training response to exercise, and mobilization of haematopoietic stem cells may play a role in this process [18]. Our finding that percentage of CD34\(^+\) cells and percentage of CD133\(^+\) cells increased in overweight and obese children following participation in our exercise programme suggests that percentage of CD34\(^+\)/CD133\(^+\) cells (endothelial progenitor cells) may mediate the tissue response to exercise.

In addition, in order to improve the English language in this manuscript it has been edited by a native English speaker (Edanz: BioMed Central recommends).

**Query 2:** In the reporting of the endothelial progenitor cells, the results were reported as a percentage of cells. Most other papers in literature report them as a number per million cells. Given that these cells are so rare, reporting them as a percentage with only 2 decimal points does not give the information that a raw number would. Also it is very interesting that the CD34\(^+\) cells in both groups increased after 2 weeks. While the p-value is reported in the exercise group it is not in the control group. It would be interesting to know the p-value in the control group as well. Some explanation for the increase in the control group is also needed.

**Response 2:** We appreciate your comments. Although several previous studies have utilized number per million cells for EPC we chose to express EPC numbers as a percentage of cells based on three previous studies (Povsic et al., 2009; Hristov et al., 2007; Steiner et al., 2005). We have added several sentences to clarify this point in the Methods section (Page 9, lines 21-24).

The percentage of endothelial progenitor cells within the lymphocyte population was defined as events triple positive for CD34, CD133 and CD34/CD133 cells. One representative blot of endothelial progenitor cells is presented in Figure 1.

[Reference]


In addition, we have modified the term “the percentage of endothelial progenitor cells” in our revised manuscript, and added the flow cytometry diagram in Figure 1, Figure Legend (Page 24, lines 3-7).

**Figure Legends:**

**Figure 1.** Gating strategies to detect endothelial progenitor cells by whole blood flow cytometry; one representative blot of endothelial progenitor cells is shown. (a) Forward/sideward scatter with lymphocyte gate (R1) indicated. Acquisition was stopped after 100,000 events acquired in R1. (b) Triple-positive cells for CD34, CD133 and CD34/CD133 were determined and described as a percentage of the lymphocyte population (R 1). Quadrants were set based on isotype controls.

![Figure 1](image)

We have added the P-values for the change in percentage of CD34⁺, CD133⁺ and CD34⁺/CD133⁺ cells in the control group. In addition, we have added several sentences to clarify this point including in the results section (Page 12, Line 16 to Page 13, Line 2).
no significant changes were observed in the control group (\(p = 0.063\)).

Within-group analysis showed that the percentage of CD133\(^+\) cells was significantly increased in the exercise group after 12 weeks relative to baseline values (\(p = 0.001\)), but no significant changes were observed in the control group (\(p = 0.068\)).

Within-group analysis showed that the percentage of CD34\(^+\)/CD133\(^+\) cells was significantly increased in the exercise group after 12 weeks relative to baseline values (\(p = 0.002\)), but no significant changes were observed in the control group (\(p = 0.084\)).

In addition, we have discussed several possible explanations for the increase (i.e. trend) percentage of CD34\(^+\), CD133\(^+\) and CD34\(^+\)/CD133\(^+\) cells in the exercise group in the Discussion section (Page 14, Lines 1-6).

Although the change in the percentage of CD34\(^+\), CD133\(^+\) and CD34\(^+\)/CD133\(^+\) cells observed in control group were not significant, there was a trend towards an increased percentage of CD34\(^+\), CD133\(^+\) cells in the control group over the study period. This could be due to the mechanisms for endothelial progenitor cells related differences may be related to the different physiologic patterns of response to growth hormone and insulin-like growth factor-1 in pubertal children (Jung et al., 2009).

[Reference]


Query 3: Several places in the discussion the authors mention that they speculate an association between the endothelial progenitor cell number and serum markers, but no statistical analysis such as a correlation coefficient is given. The paper would be stronger if these correlations were run rather than speculating at associations.

Response 3: Thank you very much for your comments. A correlation analysis was conducted and the results showed no significant correlations (please see the following
Therefore, we have modified the Discussion section. Please see the revised manuscript.

The correlations between the endothelial progenitor cells and other parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r^8$</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>0.018</td>
<td>0.927</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>0.165</td>
<td>0.393</td>
</tr>
<tr>
<td>HDL-C</td>
<td>0.028</td>
<td>0.641</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.039</td>
<td>0.841</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.292</td>
<td>0.124</td>
</tr>
<tr>
<td>Insulin</td>
<td>-0.090</td>
<td>0.641</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.158</td>
<td>0.412</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>0.210</td>
<td>0.279</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>-0.229</td>
<td>0.231</td>
</tr>
<tr>
<td>VGEF</td>
<td>0.005</td>
<td>0.979</td>
</tr>
<tr>
<td>NO</td>
<td>0.166</td>
<td>0.389</td>
</tr>
<tr>
<td>CIMT</td>
<td>-0.118</td>
<td>0.329</td>
</tr>
</tbody>
</table>

$^8$: Pearson’s correlation coefficient; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; sE-selectin, soluble E-selectin; VEGF, vascular endothelial growth factor; NO, nitric oxide; CIMT, carotid intima-media thickness.

**Query 4:** The description of the exercise methods especially in reference to the next to last sentence of the paragraph. It makes a statement about the participants training on the same equipment used for the 60% of 1 repetition max. This statement is confusing and needs some clarification within the context of the study.

**Response 4:** Thank you very much for your comments. We have clarified our description of the exercise methods in the Methods section (Page 8, Lines 10-19).
Briefly, participants were introduced to the exercise equipment and given instruction by trainers on proper lifting form and technique for the dynamic exercises: bench presses, biceps curls, triceps extensions, leg presses, leg extensions, leg curls and calf raises. The 1-repetition maximum was recorded as the maximum resistance that could be lifted throughout the full range of motion (determined in the unweighted position) using good form once. Each participant’s 1-repetition maximum strength was determined for each exercise. After determination of the 1-repetition maximum, participants trained on the same equipment at 60% of their 1-repetition maximum resistance. They performed 2 rotations of a circuit consisting of 8-12 repetitions of 7 dynamic exercises.

Responses to Reviewer 2

Key Concerns (6):

**Query 1:** The sample size of each group is relatively small. It would be useful to explain in the Methods how the sample size was determined before the study began. Which variable(s) was/were considered the primary outcome(s) to determine the power and appropriate sample size?

**Response 1:** Thank you so much for your comments. We have modified in the Methods section (Page 7, Lines 1-5).

We based the sample size that we used in our study (control; n = 14, exercise; n = 15) on previously published work (Conwell et al., 2010; Prado et al., 2010; Murphy et al., 2009). In addition, our power calculation assumed at least a 70% increase in CD34, CD133, CD34/CD133 and CIMT, with a standard deviation of 50%. For a required power of 0.9 (90%), using a two-sample t-test for comparisons, a sample size of at least 8 in each group was required.

[Reference]

Prado DM, Silva AG, Trombetta IC, Ribeiro MM, Guazzelli IC, Matos LN, Santos MS, Nicolau CM, Negrão CE, Villares SM: Exercise training associated with diet


**Query 2:** The change in carotid IMT is somewhat unexpected in just 12 weeks because this measurement can be challenging to perform and the rate of change tends to be slow. To increase confidence in the results the authors should report the test-retest variability in their research center.

**Response 2:** Thank you so much for your comments. The results of our present study are indeed inconsistent with those of several other studies (Meyer et al., 2006; Woo et al., 2004); our findings reveal that carotid IMT decreased in just 12 weeks. However, we have confirmed the validity of the analysis procedure (IMT analyses were performed by a board-certified sonographer. The test-retest coefficient of variation of IMT measurements was 0.6%. The intraclass correlation coefficient for repeated measures of the IMT is 0.6% and for echocardiographic measurements ranges from 0.5 to 0.8% in our laboratory). In addition, a recent review (Thijssen et al., 2012) has indicated that short period (i.e. 8 ~ 12 weeks) exercise training can decrease IMT in healthy subjects, as well as in subjects with cardiovascular risk factors among adults. As a result, there are several possible reasons for this inconsistency. The first is that the effect of the combined exercise (aerobic and resistance) in our current study is different from the effect of the general physical activity (i.e. swimming, sports games, walking, resistance training, agility training) reported previously (Meyer et al., 2006; Woo et al., 2004). Second, the effect of exercise on IMT may differ in different-aged populations. Therefore, prospective studies are needed to further examine the time course of changes in carotid IMT, especially in children.

[Reference]


We have added additional details of the test-retest variability regarding Carotid intima-media thickness in Method section (Page 9, Lines 3-7).

Carotid intima-media thickness analyses were performed by a board-certified sonographer. The test-retest coefficient of variation of carotid intima-media thickness measurements was 0.6%. The intraclass correlation coefficient for repeated measures of the carotid intima-media thickness is 0.6% and for echocardiographic measurements ranges from 0.5 to 0.8% in our laboratory.

Query 3: Similarly, there should be some test-retest statistics reported for the endothelial progenitor cell (EPC) measurements. The control group shows rather large increases in CD34 and CD133 that raise some concern about the stability of these outcomes. Although the corresponding changes in the exercise group were larger, the high variability should be explained. Additionally, legends for the EPC figures are too brief: please explain how the data are expressed (% on the y-axis is percentage of what?).

Response 3: Thank you so much for your comments. Unfortunately, we could not perform the flow cytometry analysis in duplicate because of cost and sample size. We have discussed several possible explanations for the increase percentage of CD34+, CD133+ and CD34+/CD133+ cells in the control group in the Discussion section (Page 14, Lines 1-6).

Although the change in the percentage of CD34+, CD133+ cells observed in control group were not significant, there was a trend towards an increased percentage of CD34+, CD133+ cells in the control group over the study period. This could be due to the mechanisms for endothelial progenitor cells related differences may be related to the different physiologic patterns of response to growth hormone and insulin-like growth factor-1 in pubertal children (Jung et al., 2009).
In addition, we have modified the term “the percentage of endothelial progenitor cells” in our revised manuscript. In accordance with your comment, we have added a sentence to clarify this point in the Methods section (Page 9, lines 21-24).

The percentage of endothelial progenitor cells within the lymphocyte population was defined as events triple positive for CD34, CD133 and CD34/CD133 cells. One representative blot of endothelial progenitor cells is presented in Figure 1.

We have modified the Figure 3, Figure 4(a), Figure 4(b) and Figure Legend.

We have also added the flow cytometry diagram in Figure 1, Figure Legend (Page 24 lines 3-7).

**Figure Legends:**

**Figure 1.** Gating strategies to detect endothelial progenitor cells by whole blood flow cytometry; one representative blot of endothelial progenitor cells is shown. (a) Forward/sideward scatter with lymphocyte gate (R1) indicated. Acquisition was stopped after 100,000 events acquired in R1. (b) Triple-positive cells for CD34, CD133 and CD34/CD133 were determined and described as a percentage of the lymphocyte population (R1). Quadrants were set based on isotype controls.
Figure 1.

Query 4: What was the timing of the post-intervention measurements relative to the last exercise session? Presumably, it could be as little as 17-18 hours if performed the morning after the last exercise, or as much as a few days. This timing is critical for understanding the changes reported. For example, as the authors cite, recent studies have shown that EPC content in the circulation increases following a single bout of exercise. This is likely due to the mobilization of existing EPCs. However, it is unclear in the present study if the increase in EPCs is due to the effect of the last exercise or adaptation to a higher level of habitual physical activity. Likewise, VEGF is known to rise and fall following exercise to the timing of the measurements is critical.

Response 4: Thank you very much for your comments. We apologize for not clearly describing the blood collection method in our original submission. Therefore, we have rewritten several sentences in the Methods section to clarify our procedure (Page 10, Lines 2-5).

Fasting venous blood samples were collected from all participants at baseline and at 12 weeks. Participants were advised to avoid physical activity for 48 hours, and to fast for at least 10 hours prior to sample collection. All samples were taken between 8 and 9 AM from an antecubital vein.

Query 5: The discussion is too long and unorganized. There needs to be better structure and flow from one topic to the next.

Response 5: Thank you very much for your comment. The discussion section has been extensively revised and reorganized. Please see the revised manuscript.

Query 6: The authors should seek assistance with English grammar and style.

Response 6: Thank you very much for your suggestion. This manuscript has been edited by a native English speaker (Edanz: BioMed Central recommends).

Additional Concerns (5):
Query 7: If group assignment was truly random, it would be rather lucky for the two groups to be so closely matched at baseline for the variables in Table 1, including the number of boys and girls per group. Was there some attempt at matching?

Response 7: Thank you very much for your comment. Yes, we did attempt to match the number of boys and girls in each group. With regard to the other variables, such as body mass and height, these were truly random in our study.

Query 8: There is no mention about whether there were any participants who discontinued the study due to illness, injury, or other reasons. This should be stated clearly.

Response 8: Thank you very much for your comment. We apologise for omitting this from the Results. In the current study, no participants dropped out for the duration of the intervention. We have added this to the Results section (Page 6, Lines 22-24).

All participants in the exercise group attended each after-school exercise session and no participants dropped out during the duration of the intervention.

Query 9: Was there confirmation of activity and diet maintenance in the control group? Similarly, there should be some assessment of the overall level of physical activity in both groups. A question that is unanswered is whether the exercise group added to the total weekly physical activity or at least vigorous activity by participating in the study programme, or if they reduced time spent in activities outside of the study programme.

Response 9: Thank you, we appreciate your comment. Participants in the control group were advised to maintain their usual daily activities during the study; however, the time spent in activities outside of the study programme was not measured. We have added this point to our discussion of the limitations of the study (Page 16, Line 25 to Page 17, Line 1).

This study had some limitations. The time spent in activities outside of our study exercise programme was not measured, and could have potentially affected the results.

Query 10: What type of dietary advice was provided to the participants? Although it is plausible that they would experience a reduction in waist circumference with 12 weeks
of exercise, the total volume of activity is unlikely to be enough to account for a decline in body mass and BMI unless they performed additional exercise outside of the programme and/or reduced dietary energy intake, especially in light of the fact that they are still growing.

**Response 10:** We also appreciate this comment. As for Query 9, the diet might be a potential confounding factor for our results. In this study we did not provide dietary assessments to the participants. However, all of the participants were in the same school and had school meals at lunch time. We also gave dietary advice to the parents in both groups. We have added this issue to our discussion of the limitations of this study (Page 17, Lines 1-2).

In addition, differences in diet might also be a confounding factor [42].

**Query 11:** There is a partial explanation for strength testing in Methods but this needs to be expanded. The results of strength tests before and after the intervention should also be provided.

**Response 11:** Thank you very much for your comment. We have provided additional explanation of our method for determining the 1-repetition maximum resistance each participant was capable of at the start of the trial. This was used to set the resistance level for each participant for the duration of the programme. Strength testing was not used as an outcome measure in this study.

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