Author's response to reviews

Title: Sedentary behaviours and its association with bone mass in adolescents: the HELENA cross-sectional study

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Author's response to reviews: see over
Dear Editor
Enclosed you will find a revision of our manuscript “Sedentary behaviours and its association with bone mass in adolescents: the HELENA cross-sectional study”. As you see, we suggest another title for the manuscript as we had to correct some analyses, and as a consequence, some of our results were slightly modified. We would like to thank the reviewers for their thoughtful and constructive comments. We have considered all of the suggestions and have incorporated them into the revised manuscript. We believe our manuscript is stronger as a result of these modifications. An itemized point-by-point response to the reviewers’ comments is presented below.
This manuscript contains material that is original and not previously published in text or on the Internet, nor is it being considered elsewhere until a decision is made as to its acceptability by the BMC Public Health Editorial Review Board.

Reviewer: MAIRENA SANCHEZ-LOPEZ

Reviewer's report:
- MAJOR COMPULSORY REVISIONS
Comment 1
In my opinion sex differences in predictors of BMC are not sufficiently discussed. Certainly, girls spent more time studying, but boys spent more time in other computer-related activities than girls. My question is: why authors did not calculate the total chair time/day, and test it for sex differences?
Answer 1
Thanks for your comment. We have computed a new variable called “total sedentary time”, which is the result of the sum of the sedentary behaviours and we have included it in our analyses (see tables 1-4). Some significant associations have been observed and
commented in the manuscript. In brief, total sedentary time was negatively associated with whole body BMC in boys (partial corr, -0.157, \(p=0.043\)) (table 2). However, significant differences disappeared after additional adjustment for lean mass (model 2) and MVPA (model 3). In girls, the total sedentary time was negatively associated with femoral neck BMC (partial corr, -0.201, \(p=0.011\)) (table 4). In spite of significant differences disappeared after additional adjustment for lean mass (model 2) (partial corr, -0.150; \(p=0.058\)) and MVPA (model 3) (partial corr, -0.152; \(p=0.056\)), there was a trend to significance in both models.

**Comment 2**
Other question, why authors did not test to control for weight in the regression models?

**Answer 2**
Thanks for the suggestion. The measurement of weight includes the fat mass, lean mass and bone mass, being the later our dependent variable. We recently published an article in which we concluded that adolescents with higher levels of adiposity have greater bone mass, but this association was explained by their higher levels of lean mass [1]. Therefore, that is the reason why we used and we would like to maintain this variable as a confounder in our analyses.

**Comment 3**
Why authors don’t show figures about prevalence of low bone mineral density in boys?

**Answer 3**
Thanks. We have commented it in the results section “Due to the importance of femoral neck in the diagnosis of osteoporosis and to the fact that regression analyses showed high and significant associations between this site and time spent studying in girls, complementary analyses were performed”. In addition, and following suggestion from Reviewer 2 we have included also a specific comment in the statistics section, as follows: “Additionally, BMC and BMD z-scores were calculated using a reference standard obtained by age and gender [2] in regions with clinical relevance in the diagnosis of osteoporosis and with significant associations in regression analyses (i.e. femoral neck). Once obtained, adolescents with low BMC [1 standard deviation (SD) below the mean]…”

**Comment 4**
The following sentence should be corrected because “time spent on console games” was not negatively associated with lumbar spine BMC as the authors say: “Our results showed that among the sedentary behaviours studied, time spent on console games and studying was negatively associated with BMC at the whole body and lumbar spine in girls” (9 pg-263-265 lines).

**Answer 4**
Thanks for the comment. Some of the results have been modified as we have transformed the sedentary variables of this study (see comment 2 of Reviewer 2). The main findings of
the study remained unchanged and we also found that the time using internet for non-study was negatively associated with whole body BMC in boys. Please, see results section.

Comment 5
5. In line 265:”……..these associations disappeared after controlling for lean mass”. And MVPA?
Answer 5
Thanks for the suggestion. The associations disappeared after the inclusion of lean mass as a covariate (Model 2). In addition, in Model 3 (Model 2 + MVPA) the association remained not significant. When we controlled for MVPA we were also controlling for lean mass. That is the reason why we said in the text “after controlling for lean mass”. However, this part of the results section has been modified as we have to correct some of them.

Comment 6
Is the prevalence of low femoral neck BMC should not be lower for those girls who spend less than 2 hours of study and achieve #3 hours of extracurricular sport than those who spend more time studying (> 2h) and made more than 3 hours of extracurricular sport? According to data from, what would be the final message?
Answer 6
Thanks for the comment. The message of the figure is that there were differences in the rate of girls having low femoral neck BMC according to their sport participation. There is a lower rate of active girls (>3h/w of osteogenic sports) having low femoral neck BMC compared to the rest of girls, independently of the cutoff selected for time of study.

- MINOR ESSENTIAL REVISIONS
Comment 1
Throughout the entire text sometimes appear “min.” and other “minutes”
Answer 1
We have corrected and used “minutes”. Thanks

Comment 2
In the statistical analysis can be read: model 0, model 1 and model 2, however in the tables and results reads: model 1, model 2 and model 3. Please homogenize
Answer 2
Thanks for catching this. Corrected.

Comment 3
What is BMD? This abbreviation should be described (line 153)
Answer 3
We referred to bone mineral density. We have described it where appropriate.
Comment 4
What is VPA? This abbreviation should be described (line 170)
Answer 4
The abbreviation has been deleted as we only mention it once in the manuscript. Thanks.

Comment 5
In line 232 should read "time of study" rather than "students"
Answer 5
Done. Thanks.

Comment 6
In line 242 lack “femoral neck” between low and BMC
Answer 6
Added. Thanks.

Comment 7
Table 1, foot table: When differences significant p values in bold?
Answer 7
Thanks. As we have modified table 1 following the comment 7 of your discretionary revisions, we have modified this as follows: * Sex differences (p<0.05). In addition, table 1 has been modified as a consequence of the comment 2 of Reviewer 2.

- DISCRETIONARY REVISIONS
Introduction
Comment 1
Not clear how increased bone mass in childhood has been associated to the frequency, intensity and type of PA. What way? This needs to be described (3 pg; 68-69 lines)
Answer 1
Some lines have been added in the introduction to clarify this as follows: “This phenomenon is related to the mechanostat theory [3, 4], suggesting that both exercise and PA could drive to a direct osteogenic effect on bone mass and an indirect osteogenic effect by increasing muscle size and strength and hence the tensions generated on bones [5].”

Comment 2
The following idea is repeated below (77-78 lines), it might seem redundant: “However, much less is known on the association among sedentary behaviours and bone health” (69-70 lines)
Answer 2
The reviewer is right. Lines 69-70 have been deleted.

Comment 3
Should include a reference to support this statement: “Therefore, a better understanding of the sedentary behaviour-bone health association is of great importance, especially on a key period as adolescence and, in regions with clinical relevance in the diagnosis of osteoporosis, such as femoral neck and lumbar spine” (86-89 lines)

**Answer 3**
Added.

**Comment 4**
I think the following sentence is not relevant in this paragraph (90-92 lines): “The Healthy Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study (HELENA-CSS) used harmonized and well standardized methods of measurement in European adolescents in 2006-07 [13].”

**Answer 4**
The reviewer is right. We have moved this sentence to the beginning of the methods section.

Statistics

**Comment 5**
1st parag. I suppose authors refer to residual in regression models, but even in this case this sentence should be placed after the description of regression models.

**Answer 5**
Done. Thanks.

Results

**Comment 6**
In girls, after adjusting for extra-curricular sport remains the negative association between study time and whole body, lumbar spine and femoral neck BMC? (2nd paragraph of the results section)

**Answer 6**
This part of the results section has been modified.

**Comment 7**
Table 1: could display the data for the total sample

**Answer 7**
We have included data for total sample following your suggestion. As a consequence, we have deleted the column with the p value between sexes and we have shown an asterisk instead (see table 1).

**Comment 8**
Tables 2, 3 y 4: What does the P stand for? Is this the P value for the partial correlation or the regression coefficient? Do we need both regression coefficient and partial correlation
results? The latter is really not contributing to any more relevant information considering the data on slope is already provided and is the more important result. To focus the reader's attention to relevant data may only be needed the table for the girls and put into text the results of boys. These tables can be revised similar to this:

**Answer 8**
This is the p value of the regression coefficient. Partial correlation is used to quantify the relationship between the two variables (bone and sedentary behaviour) while controlling for all other predictors in the model. We would like to maintain both outcomes as we consider that it could be easier for the readers to interpret the results in this way. Thanks.

**Comment 9**
Figure 1: I think it would be easier to interpret if the x-axis could be read: Time of study (h/day), and include an information to describe what is each bar. Not clear what is meant “abCommon superscripts indicate a significant differences”. Is it possible to miss a b in figure A?

**Answer 9**
Comments appreciated. Information in the x-axis has been included in bold letters (as well as in the y-axis). Columns (bars) are described at the bottom of the figure and we also included this information at the end of the manuscript file, indicating that the black columns referred to the active girls and the grey ones to the rest of girls. At each figure, when the same letter, for example, “a” is on different columns indicate that there was a significant difference between these percentages. The same for the letter “b”. We have checked the values of the figure and everything is correct. Thanks.

**Discussion**

**Comment 10**
I think the following paragraph was correctly described in the methods section so I believe it may seem redundant to be in the discussion section again: “The cut-off for time of study (low: <2 h/day and high: # 2h/day) was based on the results obtained in a recent descriptive study of sedentary behaviours in adolescents (272-274 lines)”

**Answer 10**
Deleted. Thanks.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests below
Reviewer: Janet Shaw
Reviewer's report:
MAJOR COMPULSORY REVISIONS

Comment 1
To ensure that this is a different study from the previous report by the same authors (Vincente-Rodriguez et al., 2009), the introduction (lines 82-89) should point out exactly what is different and/or expanded in this paper. I found the discussion section on lines 253-262 a bit confusing, which relates to the previous study. Are the present findings contradictory to the previous study? The present study did not find any association between BMC and sedentary behaviours in boys, but the previous study found that >3 hrs of TV watching was associated with lower whole body BMC. Is the present study based upon a different sample of boys?

Answer 1
Comments appreciated. Both the introduction and discussion have been clarified according to your comments. We have marked that the previous study of Vicente-Rodríguez et al. [6] was performed with adolescents from the AVENA study (which took place between 2000-2002). The present study, with adolescents from the HELENA study, was based on the one from Vicente-Rodríguez et al, but considering a large number of sedentary behaviours (using to this proposal a questionnaire that showed to be reliable in adolescents [7], more bone-related variables (i.e. lumbar spine and femoral neck, both used in the diagnosis of osteoporosis and measured with a newer and different equipment; DXA Lunar scanner Vs. DXA Explorer scanner) and using variables from accelerometers to control our analyses following the recent guidelines launched by the U.S. Department of Health and Human Services and other medical institutions [8] in a sample of 359 adolescents (277 in the previous study).

Introduction: “Vicente-Rodríguez et al. recently showed that adolescents from the AVENA study who watched television (TV) more than 3 hours/day…” “…In the present study, we do so but taking into account a large list of sedentary behaviours and bone mass related-variables and using the objectively measured PA as a confounder in our analyses”.

Discussion: “Little is known about the association among sedentary behaviours and bone mass in a key period such as adolescence. In adolescents from the AVENA study, Vicente-Rodriguez et al. concluded that ≥3 h/day watching TV...”

Comment 2
Were distributions for all variables assessed for normality? The SDs for some of the sedentary behaviours are quite large and in some cases, larger than the mean values. Is it possible that some of the sedentary variables are not normally distributed and require transformation of some kind prior to further analysis?

Answer 2
The Reviewer is right. We had an error when checking it for normality because we introduced new variables after a first analysis. The whole statistical analysis has been now
reviewed. Now we have transformed them, using natural logarithm and/or square root. Statistics section has been modified accordingly, as well as the tables (results) and some lines in the discussion. The main findings of the study remained unchanged and we also found that the time using internet for non-study was negatively associated with whole body BMC in boys, supporting and giving more strength to our results. In addition, table 1 has been modified and now ANOVA was performed for normally distributed variables (mean ± SD) and U Mann Whitney for non-normally distributed variables (median and interquartil intervals). Thanks for this important comment.

MINOR ESSENTIAL REVISIONS

Comment 1
There are some awkward passages throughout where some changes in verbiage would be helpful. The following lines are areas that may be edited to improve writing clarity: 64-65; 74-75; 115-116; 169-170; 181-189; 300-302. The word "showed" is used frequently; "shown" is more appropriate.

Answer 1
Comments appreciated.

Comment 2
Suggest using "participants" throughout instead of "subjects."

Answer 2
Comments appreciated.

Comment 3
Line 127 states that hours of sedentary behaviour were measured, but the units throughout are minutes. Suggest clarifying this discrepancy in units.

Answer 3
The reviewer is right. Now we do not mix hours with minutes in the text in order to not confuse the readers. The categories in the questionnaire were detailed in hours. However, for this paper, as we have estimated the minutes of each category, we consider it is better to show it in minutes. We have clarified it as follows: “A self-report sedentary behaviour questionnaire was administered during the school hours. Adolescents reported time watching TV, playing with computer games, playing with console games, surfing by internet for reasons other than study, surfing by internet due to study reasons, and studying (non-school time) for week and weekends days, selecting one of the following categories…”

Comment 4
Categories of sedentary behaviour were created. Were the categories or the continuous variables of sedentary behaviour used in the regression analyses?

Answer 4
We used the continuous variables (min/day) of sedentary behaviours (now transformed) in the regression analyses. The cut-offs for each category have been previously used [7]. A brief comment has been added in the statistic section to clarify it. “After square root transformation of the continuous variables of TV viewing...” “Relationships of sedentary behaviours (i.e., the continuous variables of TV viewing, use of computer games, console games, internet for non-study, internet for study, time spent studying and total sedentary time) with different bone mass related variables...”

**Comment 5**
Line 147 states that a spine phantom was used to calibrate the DXA equipment; the regular scanning of a spine phantom is for purposes of assuring quality control of measurement.

**Answer 5**
The reviewer is right. This has been modified as follows: “A lumbar spine phantom was used for assuring quality control of measurement as recommended by the manufacturer”

**Comment 6**
Line 203, "mean - 1SD" is used to define low BMC. As written, I am unable to construe exactly what this means. A more detailed description of this low BMC variable would be very helpful.

**Answer 6**
Now we have used “1 standard deviation (SD) below the mean” the first time it appears and “1SD below the mean” in subsequent appearances.

**Comment 7**
Reference 9 is incomplete.

**Answer 7**
Corrected

**DISCRETIONARY REVISIONS**

**Comment 1**
On line 114, "objectively measured PA and sedentary behaviours" reads as though both PA and sedentary behaviours were measured objectively, when only PA was measured objectively. Suggest "objectively measured PA and self-report of sedentary behaviours."

**Answer 1**
Corrected. Thanks.

**Comment 2**
Lines 165-177, references to justify the accelerometry cut-points are from previous studies using the same cut points. The reader may appreciate the primary references from which
these cut points are justified for moderate and vigorous PA by accelerometry in adolescents.

**Answer 2**
Thanks for the suggestion. References 24 and 25 have been replaced.

**Comment 3**
The first sentence of the statistics section (line 192) could be re-written to form a more descriptive topic sentence.

**Answer 3**
This sentence has been modified also considering comment 5 (discretionary revisions) from Reviewer 1.

**Comment 4**
After reading the results, I understand why time of study was singled out in the analysis of the girls' BMC. However, at first glance, I had difficulty understanding why this analysis was being done. Perhaps some explanation for this additional analysis would be helpful to other readers.

**Answer 4**
We have included some information in this regard in the statistics section, as follows: “Additionally, BMC and BMD z-scores were calculated using a reference standard obtained by age and gender [2] in regions with clinical relevance in the diagnosis of osteoporosis and with significant associations in regression analyses (i.e. femoral neck). Once obtained, adolescents with low BMC [1 standard deviation (SD) below the mean]...”

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.

**REFERENCES**


