Author's response to reviews

Title: Low-dose combined oral contraceptive use is associated with lower bone mineral content variation in adolescents over a 1-year period

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Author's response to reviews: see over
Dear Editor and Reviewers,

Enclosed please find the revised version of our manuscript entitled "Low-dose combined oral contraceptive use is associated with lower bone mineral content variation in adolescents over a 1-year period", which we are resubmitting for consideration for publication in BMC Endocrine Disorders.

Below is a list of our answers to the minor comments of the reviewer Prof. Jan Stepan. We thank the editor and reviewers Prof Nihal Hatipoglu and Prof Jan Stepan for their valuable suggestions and comments that helped improve our manuscript and remain at your disposal to clarify any points that have not been answered to your satisfaction.

Sincerely yours,

Tamara B. Lederer Goldberg, MD, PhD and authors

Reply to minor comments

Comment 3. Please, state the short-term in-vivo precision errors for lumbar spine and total body BMC; state the long-term precision error using the Hologic phantom. Did daily scanning of a phantom show absence of machine drift during the study?
First reply: The CV was estimated from repeated measurements for all regions mentioned (lumbar spine and total body). With the results in hand, CVs of 0.6% and 1.3% were obtained for the lumbar spine and for the whole body, respectively.
Suggestion: Please, make sure that the CV was estimated in the adolescent subjects according to the ISCD recommendation. Please, consider to state absence of machine drift during the study.

Dear Prof. Stepan, we thank you again for your suggestions and to leave no doubt regarding the precision and accuracy of the densitometry exams, we added the following text:

The DXA instrument was calibrated by daily scanning of a hydroxyapatite spine phantom. Machine drift was not observed during the study. The CV was estimated from repeated measurements (twice) obtained from 30 patients representative of the clinic’s patient population for all regions mentioned (lumbar spine and total body) after each patient had
been repositioned before scanning. With the results in hand, CVs of 0.6% and 1.3% were obtained for the lumbar spine and for the whole body, respectively (Crabtree et al., 2014). (Manuscript: line 158 to 164)

According to:


Recent data have shown that the precision of DXA in adolescents (expressed as CV) is similar to published values for adults, but the CV obtained for young children was higher (45).

Comment 4. Was the sampling and storage done for the measurement of biochemical markers?
Reply: Yes, they were stored for the measurement of bone biomarkers (OC, BAP and S-CTX).
Suggestion: Please consider completing the article with biomarker data. This may provide an interpretation of the BMC data (expansion of the bony size)

Dear Prof. Stepan, we would very much like to complete the article with this information. However, although the samples were collected and the serum was separated and stored in a freezer at -70°C, we are waiting for the approval of funds from the São Paulo State Funding Agency (Fundação de Amparo a Pesquisa do Estado de São Paulo - FAPESP). The fund estimated for purchasing the kits for the measurement of OC, BAP and S-CTX is more than US$ 5,000.00, an amount which we do not have at this moment. Hopefully, we will obtain these results at some point, but we would like to publish them in the future to justify further studies and new grants.

Final comment (suggestion).
The subjects in this study were measured for height with a wooden height gauge with 0.1-cm accuracy. Therefore, it is quite surprising that in both groups height did not vary between the measurements taken at baseline and after 12 months. The authors argue that as the outcome is the evolution, baseline and final (after 12 months), BMC data were combined and the outcome that expresses evolution was thus created. Was the same statistical approach applied to change in body height? In subjects using COC reduced bone growth rather than lower BMD is expected.

Dear Prof. Stepan, we thank you for the interesting comment. Again, we emphasize that the girls participating in the study were in late puberty and therefore had already undergone the growth spurt, surpassing growth deceleration when COC was introduced (the median time interval between menarche and starting COC use (gynecological age) was 48 months). However, they should still incorporate bone mass, an event that occurs after peak height velocity (Vatanparast & Whiting, 2005). The text of these authors is cited below:
"Bone Mass Development"

**Time of peak bone mineral content velocity (PBMCV)**

"The dramatic increase in bone mineral content (BMC) during adolescence is a function of maturation (6). Approximately 90-95% of an adult’s bone mineral is achieved by the end of adolescence and the final 5-10% may be added over the next 10 years (7,8). BMC is closely correlated with height in children until the occurrence of the adolescent growth spurt. Peak height velocity (PHV) is achieved at age 11.8 y in girls and 13.4 y in boys and it is attained ~ 2 years earlier in girls compared to boys (9,10). There is over a 0.7 year lag time between PHV and PBMCV (Figure 1) (9-11)."

We hope to have answered this important question.

Below is a table reporting the height data, showing no significant difference after the introduction of COC. However, we believe that for publication there would be excess results to be presented to the readers. We thus left in the text the sentence to which it refers (Manuscript: lines 192 to 196).

The variables were obtained at baseline and at 6 and 12 months after COC use.

**COC Users (n=35)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>6 months</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.75 a</td>
<td>12.00</td>
<td>19.50</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.20 a</td>
<td>41.00</td>
<td>73.40</td>
</tr>
<tr>
<td>Bone age (years)</td>
<td>16.50</td>
<td>14.00</td>
<td>18.00</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.59</td>
<td>1.49</td>
<td>1.67</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.88 a</td>
<td>16.63</td>
<td>27.82</td>
</tr>
<tr>
<td>BMI (percentile)</td>
<td>60.23</td>
<td>2.66</td>
<td>94.38</td>
</tr>
</tbody>
</table>

**Notes:** Friedman test followed by Dunn test.
- Same lower case letters indicate no significant differences between groups.
- Different lower case letters indicate significant differences between groups (p<0.05)

In the control group, the mean and median height was 1.62±0.06 and 1.64 m, respectively, at baseline and 1.63±0.05 and 1.64 m after one year.