Author's response to reviews

Title: Hyperadiponectinemia enhances bone formation in mice

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Author's response to reviews: see over
To Wenhan Chang, M.D.

Thank you for your instructive comments.

In accordance with your suggestions, we have changed the manuscript as follows.

Minor Essential Revisions

1. Page 6, Line 1:

No, we didn’t. We performed “toluidine blue“.

2. Page 6, Line 6:

Yes, we did. We have changed the wording to “the bone formation rate and mineral apposition rate in the secondary spongiosa”. Please see page 6, line 2.
To Lilian I. Plotkin, M.D.

Thank you for your instructive comments.

Minor Essential Revisions:

1. Point 2 of original review (Introduction):

In this paragraph, our intention was to indicate the conflicting opinions regarding the effects of circulating adiponectin on bone metabolism, although this may have misled the readers, as you suggest. Therefore, we have removed this paragraph and simplified the wording. Please see page 4, lines 13-14.

2. Point 7 of original review (Results):

The other reviewers indicated that immunohistochemistry does not add much to the manuscript, since quantitative assessment of bone formation was performed by histomorphometry. Therefore, we have deleted the description of the immunohistochemical technique, and the related results, from the “Materials and Methods” and “Results” sections.
To Urszula Iwaniec, M.D.

Thank you for your instructive comments.

In accordance with your suggestions, we have changed the manuscript as follows.

Major Compulsory Revisions:

1. We have changed “osteoblastgenesis” to “bone formation” throughout the manuscript. Please see the Title (page 1, line 1), Abstract (page 3, line 12), and Discussion (page 10, line 18).

Similarly, we have changed “osteoclast function” to “osteoclast number”. Please see page 10, line 15, and page 11, lines 13-14.

2. Methods:

   - There were 8 mice in each group (Lines 11 and 13 representing Ad-Tg mice, and their WT littermates): the right femur was used for BMD analysis, and the left tibia for bone histomorphometric evaluation. A total of 24 mice were sacrificed and analyzed in this study. This has been added to the “Methods” section. Please see page 5, lines 13-16.
- The area of interest in the proximal tibia was secondary spongiosa 1.26 mm distal to the growth plate, excluding primary spongiosa in an area 0.3 mm from the growth plate and 0.15 mm from cortical bone.

- Mice were sacrificed at 24 hours after the last calcein label injection.

- This was our mistake, and has now changed it. Please see page 6, line 2.

- We did not obtain any data for the growth plate. As mentioned above, this was our mistake.

- MS/BS means the mineralization ratio. We have added details to the Figure legends section. Please see page 17, line 14.

- Detection limits, and the intra- and interassay variation for each assay were as follows:

  TRAP: sensitivity, 0.1 U/L; intra-assay variation, 6.5%; interassay variation, 8%

  RANKL: sensitivity, 5 pg/mL; intra-assay variation, 2.2-8.1%; interassay variation, 6.2-7.9%

  OPG: sensitivity, 4.5 pg/mL; intra-assay variation, 4.3-7.9%; interassay variation, 6.9-7.4%

  Adiponectin: sensitivity, 0.246 ng/mL; intra-assay variation, 2.5-4.7%; interassay variation, 5.8-6.8%
Osteocalcin: sensitivity, 1 ng/mL; intra-assay variation, 6.0%; interassay variation, 8%

- Yes, they were.
- The term “cancellous area” meant “spongiosa area”.

3. Results:

- We have added BMC data to Figure 2B. Please see this figure.
- We have adjusted the data to 1 decimal place. Please see the new Table 2.
- As you suggested, Figure 3 has now been deleted. In addition, we have changed “more than double” to “significantly increased”. Please see page 8, lines 21-22.
- We have added the bone formation rate (BFR/BS) to Table 2.
- We have deleted Figure 4, and therefore removed the explanation of the immunohistochemical technique and the related results from the “Methods” and “Results” sections.

4. Discussion

As mentioned above, we have changed the wording to “osteoclast number”. Please see page 10, line 15, and page 11, lines 13-14.