Reviewer’s report

Title: No changes in levels of bone formation and resorption markers following a broad-spectrum antibiotic course

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Reviewer: Viola Guardigni

Reviewer's report:

In this manuscript, Mikkelsen and colleagues tested the hypothesis that antibiotic-induced changes in gut microbiota might have effect on bone metabolism in humans. For doing this, the authors analyzed serum/plasma levels of serotonin, sex hormones, GLP-2 and bone turnover markers (BTMs) before and after a short broad-spectrum antibiotic course. In mice, there are evidence that gut microbiota can influence bone remodeling, but results are conflicting.

The study has a rationale and has been carried out with quite an adequate approach: multiple study visits at precise time-points have been conducted with simultaneous analysis of both plasma and stool samples.

The major limitations are the small sample size (only 12 subjects) and the lack of a comparison group, along with the use of surrogate markers of bone turnover, and the authors acknowledge all of them. The absence of a comparison group (e.g. including men with bone alteration) would have definitely increased the strength of the study.

Despite this, the paper address an important topic in the field, given the increasing understanding that gut microbiota plays a relevant role in many physio-pathological process and it will be to be taken into account in the future management of several clinical conditions, such as bone metabolism alterations. The manuscript highlights the importance of developing knowledge on the relationship between gut microbiota and bone metabolism in humans, in order to modelling microbiota to have effect on BMD. However, some relevant points about dysbiosis, gut microbiota characteristics and analysis are lacking/not clear.
Major comments:

- Antibiotic-induced dysbiosis (i.e. a shift in the gut microbial community composition) is a well-described disorder and has been linked to different conditions like age or HIV-associated systemic inflammation.

The authors do not mention that and do not consider in the discussion that antibiotics use may also alter intestinal homeostasis, stimulating systemic inflammation with a possible detrimental impact also on bone loss. They should mention this and explain the study approach in relationship to this point. Do the authors hypothesize that reducing microbiota bacterial diversity can have a good impact on the bone metabolism? By which mechanism?

- In the background the authors should better define what they mean when they mention "microbiota" or "gut bacteria", in terms of what types of bacteria were mostly represented or at least say if microbiota was "normal" in bacterial species richness and diversity. (e.g. line 50, 52, 56, 60).

- In laboratory methods and statistical analysis section: methods on stool collection and stool microbiota analysis are lacking, as well as reference to statistical approach for microbiota analysis.

Minor comments:

- Line 48: "Thus" should be replaced with "Indeed"

- Line 57: "in 20 weeks old mice (C57BL/6)" should be moved right after "estrogen deficiency"

- Line 73: They should write "microbiota changes"

- Authors should mention for clarity in the methods that the chosen antibiotics (vancomycin, gentamicin and meropenem) are nonabsorbable when administered orally and explain why they chose them.
Are the methods appropriate and well described?

If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?

If not, please specify which controls are required in your comments to the authors.

Unable to assess

Are the conclusions drawn adequately supported by the data shown?

If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?

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I am able to assess the statistics

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