Author’s response to reviews

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

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Author’s response to reviews:

Reviewer reports:

Viola Guardigni (Reviewer 1): 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?

Response:

We agree that antibiotics may induce intestinal dysbiosis, which in turn can potentially affect bone metabolism negatively. However, our aim with this study was rather basic: to test in humans, the association between gut bacteria, bone turnover and pathways proposed to be involved in bone/microbiota crosstalk (serotonin, sex hormones, gut hormones, inflammation). We found no effect of antibiotic gut bacteria eradication on bone turnover markers or any of the above pathways in healthy young males, but as our study did not include a detailed (sequenced-
based) microbiome characterization nor further immunological measures, we don’t feel that a discussion of microbiome/immune system interplay is justified in the manuscript.

- 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).

Response:

A clarification of the term microbiota has now been added (line 47). Data from stool cultivation analyses has been previously published and we prefer not to further comment on these findings in the present manuscript. Unfortunately, the stool cultivation technique used does not allow characterization of microbial diversity and richness.

- 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.

Response:

Thank you for this comment. We deliberately refrained from including the information because it can be found in our previously published work. It is stated in the revised manuscript that the information is available in reference number 16.

Minor comments:

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

This suggestion has now been accepted.

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

Response:

The sentence has now been revised.

- Line 73: They should write "microbiota changes"

Response:

We agree, this has now been added.

- 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.

Response:

Thank you for identifying this oversight. We agree, this is now added.
Lilian Plotkin (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

The current manuscript describes the effect of antibiotic administration on metabolic and bone resorption/formation markers in adult men. The study is interesting, and potentially important to understand the consequences (or lack thereof) of antibiotic administration. The results are largely negative, leading to the conclusion that bone remodeling is not affected by the antibiotics. The study design has limitations, including the lack of a non-antibiotic control group. Nevertheless, the study has value, and only minor issues should be addressed.

Specific comments:

1- Authors should avoid using the BTM abbreviation, which is not a common one and might not familiar to the readership of the journal.

Response:

We agree and have revised accordingly.

2- The figure is disorganized, and it is hard to understand what follows under A and what under B. It might be better to separate the 2 panels into 2 figures.

Response:

The figure has now been separated.