Author’s response to reviews

Title: Low bone turnover in premenopausal women with type 2 diabetes mellitus as an early process of diabetes-associated bone alterations: a cross-sectional study

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Author’s response to reviews:

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Dear Editor,

We thank you for reviewing our manuscript entitled, “Low bone turnover in premenopausal women with type 2 diabetes mellitus as an early process of diabetoporosis: a cross-sectional study”. We appreciate that the Editor considered that the work with the necessary revisions is still potentially acceptable for publication.

In the current submission, we have incorporated the input of the editor and reviewers and revised the manuscript. We believe our revised manuscript has clearly improved. Please find our point-to-point response to the comments below. Changes in the manuscript can be identified by track changes.

Sincerely Yours,

On behalf of all authors,

Melisa D. Puspitasari
Reviewer 1 (Giuseppina Russo)

This cross-sectional study aims to assess bone turnover markers (P1NP and CTX) in 41 premenopausal women with T2DM and 40 premenopausal women without DM. This aim of the study to explore premenopausal alteration in bone turnover in T2DM is intriguing because of the mixed literature and the higher rate of postmenopausal cohorts that have been studied so far. Conclusions are appropriate to research design and results, and they may have implications for future research. The results are interesting but some minor flaws should be addressed before publishing the manuscript.

Our response: We thank Reviewer 1 for the overall critical input. Please find below our point-by-point responses.

1) The term Diabetoporosis is not widely accepted. I suggest to substitute it with bone alterations linked to diabetes or put as "Diabetoporosis".

Our response: We are aware that the term diabetoporosis is not yet widely accepted, as this was only recently proposed and there is a paucity of data on the particular subject. We agree with the reviewer that the term diabetes-associated bone alterations would be more suitable for our manuscript. We have therefore revised the term throughout our manuscript (page 1 line 4 and page 3 line 84).

2) Discussion section should be improved including the following:
   a) Small sample size should be acknowledged among the limits of the study in the discussion section.

   Our response: We have previously stated this as a limitation but have now revised the statement for clarity (page 13 line 311).

3) The lack of US and/or DEXA measurements as well as hormonal status should also be acknowledged among the limitations of the study, and properly discussed.
Our response: We have now revised the limitations of our study to include points as suggested by Reviewer 1 (page 13 line 312).

4) Ethnic implications of studying an Indonesian population should also be addressed (i.e., effects of BMI on bone strength etc.)

Our response:

This study is performed in the Indonesian population with all subjects being of Malay ethnicity. Some studies indicate that there were inherent differences in bone turnover between the different ethnicities (Leder et al., 2007, de Papp et al., 2007, Henry and Eastell, 2000, Holvik et al., 2006, Ardawi et al., 2010, Finkelstein et al., 2002). However, others suggest that ethnicity per se is not important, and that apparent ethnic differences in rates of bone loss were largely explained by differences in body weight (Finkelstein et al., 2008, Vasikaran et al., 2011, Cifuentes et al., 2003). Studies also reported that levels of P1NP and CTX may be influenced by BMI (Weinbrenner et al., 2003, Kučukalić-Selimović et al., 2013) which did not differ among groups in our study. We have incorporated this in our discussion on page 12 line 289.

5) Also gender-specific implications of hypoglycaemic drugs should be properly addressed (see also Russo GT et al. Fracture Risk in Type 2 Diabetes: Current Perspectives and Gender Differences. Int J Endocrinol. 2016;2016:1615735. doi: 10.1155/2016/1615735. Epub 2016 Dec 4). At this regard, it would be interesting to include the list of currently used hypoglycaemic drugs in table 1 (not only insulin users).

Our response: We thank the reviewer for the suggestion to include a discussion on gender-specific implications of hypoglycaemic drugs. We have added the list of currently used hypoglycaemic drugs in table 1 as the reviewer suggested. Gender differences in BMD, bone structure and the risk of fracture in individuals with diabetes have been acknowledged, with women being particularly at risk for DM-associated bone alterations (Russo et al., 2016). It remains largely unclear to what extent hypoglycaemic drugs are gender-specific. Only thiazolidinediones were shown to have negative effects on bone metabolism in women (Glintborg et al., 2008, Berberoglu et al., 2007, Grey et al., 2007). Although our study only involved female subjects, there were none who were on thiazolidinediones. We have included this remark in the discussion section on page 12 line 298.
Guillaume Mabilleau, Ph.D (Reviewer 2):

I read with attention the manuscript entitled "low bone turnover in premenopausal women with type 2 diabetes mellitus as an early process of diabetoporosis: a cross-sectional study". The main goal of this study was to assess whether changes in bone turnover occur early in T2DM by investigating the circulating levels of CTx and P1NP in premenopausal women.

Overall, the manuscript is well written and pleasant to read. The english syntax is ok. However, the idea that bone turnover markers are altered in T2DM premenopausal women in not new and has already been investigated by Christensen and Svendsen (Osteoporosis Int, 1999, 10: 307-311) who reported a reduction in bone resorption and no effects on bone formation. The difference between the current and the published studies should be clearly stated in the introduction.

Our response: We thank Reviewer 2 for the important input on the study by Christensen (Christensen and Svendsen, 1999) that we previously overlooked. The study by Christensen examined bone turnover in premenopausal and postmenopausal women with T1DM and T2DM, and this is indeed important to include in our introduction. However, as the reviewer pointed out, there were differences between this older study and ours. The Christensen study only measured osteocalcin as a marker of bone formation and did not show any statistically significant difference in osteocalcin levels between the premenopausal women and the reference population. Thus, our study remains as the only study addressing a decrease in bone turnover markers in premenopausal women with T2DM. We have revised our introduction to include this important reference in page 4 line 115.

Major concerns:

- Page 3, line 80: the authors state that diabetoporosis is characterized by "microarchitectural changes" without citing them. Furthermore, lines 88-89 they reported changes in cortical porosity. It should be stated here that changes in cortical porosity is the only significant microarchitectural changes observed in a subset of Afro-American T2DM population and is not often observed in the rest of this subpopulation or Caucasian. As such, I would recommend being more precise when reporting microarchitectural changes and rather state that it is indeed cortical porosity.
Our response: We thank reviewer 2 for this input. We have subsequently remove the term "microarchitectural changes" (on page 3 line 84) and now write this more precisely in the introduction as cortical porosity when applicable and incorporate the relevant references (page 3 line 93). We have also now cited the study by Yu and colleagues which reported higher cortical porosity in the African-American women (Yu et al., 2015). Although this group also reported that healthy, non-DM African-American women had lower cortical porosity compared to Caucasians (Putman et al., 2013), to our knowledge no other studies compared cortical porosity between the different races.

- The assessment of bone resorption is made by investigating the circulating levels of CTx. CTx corresponds to a fragment of the triple helix of collagen that sustain some cross-links. However, cross-linking of the collagen matrix is altered and reduced in T2DM and as such, investigation of CTx might be problematic to draw any conclusions in T2DM as lower value may represent same extent of bone resorption but less cross-linking of the collagen matrix. This is one of the reasons why assessment of bone turnover is not massively reported in the literature. This should be discussed in the discussion as a limitation to this study.

Our response: Reviewer 2 raised the concern on the reliability of CTX, a classical marker of bone resorption, in assessing T2DM individuals. The possible effects of chronic hyperglycemia on the reliability of CTX as a marker of resorption is currently unknown. However, while cross-linking of the collagen matrix is altered in T2DM, it remains unclear whether they are reduced (Garnero, 2012, Yamagishi et al., 2015). CTX is the product of type I collagen, and there is the possibility that the degraded type I collagen might have already been glycated in diabetic patients. However, to the best of our knowledge, there is no method that can differentially measure glycated CTX from unglycated CTX. A study by Reyes-Garcia reported lower bone resorption as depicted by lower CTX in concurrence with lower tartrate-resistant acid phosphatase 5b (TRAP5b) in T2DM individuals, suggesting that indeed lower bone resorption occur in T2DM. To date, numerous studies on bone metabolism in T2DM have utilized CTX to assess bone resorption (Starup-Linde et al., 2014, Reyes-Garcia et al., 2013, Jiajue et al., 2014, Farr et al., 2014, Manavalan et al., 2012, Movahed et al., 2012, Zinman et al., 2010, Bunck et al., 2012, Gruntmanis et al., 2010, Bilezikian et al., 2015). However, as this is indeed a potential concern, we have now incorporate this in the discussion section on page 11 line 265.

- The alcohol consumption and smoking status of the premenopausal women enrolled in the study should be reported, as these two factors are known to lead to osteoporosis.
Our response: None of the subjects in this study consume alcohol or smoke, and this is common in Indonesia due to cultural reasons. We have included this information in page 7 line 183.

- Page 9, line 210-212: the authors stated that the low bone turnover lead to higher bone mineralization that is not captured by measuring BMD alone. This statement is erroneous. The lowest the bone turnover, the highest maturation, and hence mineralization, of the bone matrix components. As such, an increase in bone mineralization might be perceived as an increase in BMD although the bone quantity is unchanged. This is one of the hypothesis to explain why BMD is increased in T2DM.

Our response: We thank Reviewer 2 for this critical input. Indeed, low bone turnover, as seen in T2DM individuals, may reflect a slowing down in the process of replacing older, more densely mineralized bone with younger, less densely mineralized bone, leaving a relatively heightened state of bone mineralization as shown by increased BMD (Seeman, 2003). We have also incorporated additional explanation as to why BMD is increased in T2DM. Data showed that the higher BMD in T2DM was not associated with bone geometrical instability or bending strength (Oei et al., 2013). However, a study by Burghardt and colleagues used a high-resolution peripheral quantitative computed tomography that enabled assessment of volumetric BMD (vBMD) independently in cortical and trabecular compartments of the bone. T2DM subjects, especially those with previous fracture, showed marked levels of intracortical porosity with an extremely dense trabecular bone in the peripheral region adjacent to the cortex, an assessment that is not captured by conventional measurement of BMD. The authors concluded that this a potential explanation for the inability of BMD measures to explain increased fracture incidence in patients with T2DM (Burghardt et al., 2010). Another study also reported lower cortical vBMD in T2DM individuals (Samelson et al., 2017). We have included this in the discussion on page 10 line 237.

References


