World Journal of Diabetes Editorial Team

30 April 2022

Answers to Reviewers

Dear Sirs,

On behalf of the research group, I would like to express our deep appreciation to reviewers for their prompt and productive review of the manuscript. We have made every effort to revise the manuscript accordingly. Here we provide a step-by-step response to the reviewer`s comments and suggestions.

Reviewer 1	
1. Title: As the study design is cross-sectional and therefore not able to establish causality, the authors should consider changing the word "affecting" to "associated with". i.e., Factors Associated with Trabecular Bone Score in Postmenopausal Women with Type 2 Diabetes and Normal Bone Mineral Density	We certainly agree with this point. The title was corrected.
2. Abstract, Methods: The words "POC curves" should be "ROC curves".	The typos have been fixed.
3. Abstract, Results: Numerical results, such as odds ratio and p values, should be provided.	We have included statistical parameters in the Abstract.
4. Abstract, Conclusion: The conclusion should not merely a repeat of the sentences in the Results section. The authors may want to use what they have indicated in the Core tip section.	We have updated Conclusion as recommended.
5. Methods: As a longer duration of type 2 diabetes is generally associated with increased fracture risk, is it possible to include and adjust for the potentially confounding effect of duration of type 2 diabetes?	Thank you for the suggestion. We tested this hypothesis in multiple linear regression analysis and in logistic regression. Initially, we have checked if all assessed clinical and laboratory parameters are significant. However, with backward elimination procedure, duration of diabetes, as well as age, age at menopause and time since menopause, HbA1c, and eGFR, were excluded from the models as non-significant.

6. Methods: Were the chronic diseases listed in the exclusion criteria ascertained from diagnosis on the medical record of eligible participants? For example, for the criteria "any kind of malignancy", is there a specific period or just "ever diagnosed with any kind of malignancy"?	A detailed study of medical history as well as clinical and laboratory examination were performed in all patients to rule out the risk factors for secondary osteoporosis as non-inclusion criteria. Those ever diagnosed with any kind of malignancy were not included. We have refined this non-inclusion criterion to eliminate ambiguity.
7. Statistical Analysis (page 8): "Statistics 13.0" should be indicated as "Dell Statistica 13.0 (Dell Software, Aliso Viejo, CA, USA)"	It was corrected.
8. Statistical Analysis (page 8): More details should be provided for the sample size calculation, including the choice of effect size.	The information was added.
9. Statistical Analysis (page 8): IBM SPSS should be cited as "IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp.)".	We have edited it as indicated.
10. Results: The description of the variables in Table 1, 2, and 3 should include p values (if significant).	P-values were included in the tables.
11. Results: The description of the regression models should include p values and odds ratios, as appropriate.	The information was added.
12. Results: The authors should consider adding a new table to show the results of the multivariate stepwise regression analysis. Only variables that were significantly associated with a decreased TBS should be retained in the final model and shown in the table.	Thank you for this suggestion. We have added the table with the results of multivariate stepwise regression analysis.
13. Results: Please explain why the multiple logistic regression analysis was not performed with a variable selection procedure, such as backward elimination.	Thank you for your note. Actually, we used backward elimination procedure in multiple linear and logistic regression analysis.
14. Discussion: TBS was analyzed as both a continuous and binary variable using linear regression and logistic regression, respectively. In the Discussion and conclusion, the authors mixed the findings from both analyses as if they were from a single regression analysis. The two results should be explained separately because they are based on different outcome. The authors should explain the advantage and	We unified the results of the two modeling methods in new version of the manuscript. Both linear regression and logistic regression identified height, android and gynoid fat as the most significant factors associated with a decrease in TBS. We believe that the use of two methods of regression analysis considering TBS as a continuous and binary variable provides more detailed

limitation of treating TBS as a continuous and binary variable. For example, the choice of cut-off for TBS value was chosen according to the results of a meta-analysis. However, the 95% CI for it was 1.21–1.42. A different set of significant variables might emerged with a slight change in the cut-off value.	quantitative information about the contribution of each factor to bone microarchitecture. The issue of the TBS cut-off point is important for clinical practice. In the revised manuscript, we have provided a more detailed rationale for choosing the cut-off point for this parameter.
15. Discussion: "HU Moon et al. have shown that TBS increase as visceral fat mass decrease in men and women with T2D [24]" should be "Moon et al. have shown that …"	It was corrected.
16. Conclusion: It is mentioned that "older age, greater height and lower body weight, as well as central adiposity" were the significant predictors. However, only BMI was identified as the risk factors of decreased TBS based on ROC analysis. It is not clear why lower body weight was mentioned.	It was corrected.
17. Table 1, 2 & 3: Please add a new column showing the exact p values, regardless whether it is significant, for all variables.	The tables were updated as recommended.
18. Table 1, 2 & 3: The footnote "TBS <1.31, group of individuals with TBS <1.31"≤1.31, group of individuals with TBS ≤1.31" should be "TBS	The tables were updated as recommended.
Reviewer 2	
Methods	
1. The authors state a power calculation was done, however the minimum sample size determined using the power calculation is not stated.	Thank you for this note. We have added information about the sample size calculation in the revised version of the manuscript.
Results:	
1. Paragraph 4, please include the types of fractures. It would be useful to add fractures into Table 1.	The data were added into the text and Table 1.
2. It would be useful to include a table for the results of the stepwise multivariate linear regression analysis, as well as the logistic regression analysis.	Thank you for this suggestion. We have added a table with the results of linear regression analysis in the revised version of the manuscript.
3. In the model of multivariate stepwise regression analysis, the authors state that age, age since menopause, gynoid fat mass and eGFR were significant predictors of TBS (results paragraph 7). However, the	Thank you for this note. When revising our data, we sought to unify the results of different types of analysis. In ROC-curve analysis, we were unable to find a reliable cut-off point for android fat.

ROC analysis included height, BMI and the android / gynoid fat mass ratio. What was the reasoning for choosing these parameters for ROC analysis, when they were not found to be significantly associated with TBS in multivariate stepwise regression analysis?	Therefore, we calculated the cut-off for the android/gynoid ratio. We also included body mass index in the ROC analysis, as a more available parameter compared to the body composition.
4. Regarding logistic regression analysis, were all factors included in the initial stepwise logistic regression analysis, or only those listed in Table 5? If all factors were included, then these should be included in Table 5 (either in the footnotes, or in the table itself). If not, what is the reasoning for including only certain factors in the logistic regression analysis?	Initially, all studied clinical and laboratory parameters were checked if being significant in logistic regression model. After backward elimination procedure, only statistically significant factors were retained.
Discussion	
1. First paragraph, the authors state that 'older age, height, lower BMI and gynoid fat mass, higher android fat mass and greater android / gynoid fat mass ratio' contribute to TBS decrease. However, when adjusted by multivariate linear regression, only age, age since menopause, gynoid fat mass and eGFR were associated with TBS. Different factors were found in logistic regression analysis. Given adjusted analyses were done, it is inaccurate to state as a summary the univariate analysis results, as these are likely to be confounded by other factors.	Thank you for this note. We have unified the results of the two modeling methods in new version of the manuscript. Both linear regression and logistic regression analyses identified height, android and gynoid fat as the most significant factors associated with a decrease in TBS. We have updated the Discussion section according to the obtained results.
2. Paragraph 3, the authors state 'We identified older age and younger age at menopause as factors associated with lower TBS values, although we were unable to establish cut-off points for these parameters'. Did the authors attempt to determine a cut-off value, if so why could a cut-off not be established?	The cut-off points for these parameters were not significant after calculation OR and 95% CI in ROC-analysis. We removed this sentence from the Discussion.
3. Paragraph 5, authors state 'At the same time, it is believed that vitamin D deficiency can be a causative factor for insulin resistance and associated disorders.' The data linking vitamin D deficiency to insulin resistance is still inconclusive and causation has not been established. I think the authors should include comments regarding the	We fully agree with this remark. We have modified this sentence as follows: At the same time, it is believed that vitamin D deficiency can be associated with insulin resistance and related disorders.

uncertainty here, or else leave this line out.	
4. Paragraph 6, the authors state that 'we were unable to identify HbA1c as a risk factor for a decrease TBS, we cannot exclude the role of hyperglycemia in the deterioration of bone microarchitecture'. Can the authors include some comments about why this might be? For example, could the association be U-shaped, might glycaemic variability rather than HbA1c be associated with bone? A number of studies have been published on this issue, and would be important to include here.	Thank you for bringing up this important issue. In our study, HbA1c was only slightly higher in patients with TBS <1.31. Most of the patients had long-term diabetes and non-target glycemic control parameters on combined antidiabetic therapy. These factors could modify the effect of hyperglycemia on TBS. Besides, single HbA1c measurements were included in the analysis. Therefore, the effect of metabolic memory on bone structure cannot be ruled out. We included this explanation in the Discussion section. We have also included data from other studies demonstrating the relationship between glycemic control and TBS in the revised version of the manuscript.
5. Paragraph 7, authors could reference studies using TBS adjusted FRAX in diabetes, as it appears that the adjusted FRAX still under-estimates fractures in these patients (Eg article by Leslie, 2018).	Thank you for pointing out this important issue. We have cited the study by Leslie et al. (2018) in the revised version of the manuscript.
6. Limitations: The obvious major limitation of this study is the observational nature, and single centre site. The authors state theirs is the first study investigating risk factors for impaired bone microarchitecture in post-menopausal women with type 2 diabetes and normal bone mineral density. I think it is important to mention 'microarchitecture by TBS' here (see point below).	We have modified this sentence as follows: At the same time, as far as we know, this is the first study estimating the risk factors for impaired bone microarchitecture assessed by TBS in postmenopausal women with T2D and normal BMD.
7. Throughout the paper, no mention is made of HRpQCT. HRpQCT is considered the gold standard for non-invasive assessment of bone microarchitecture. While TBS is more available and less expensive, I think it is important for authors to acknowledge this technology. Studies have been done examining HRpQCT in diabetes.	Thank you for this suggestion. We have added information on methods for assessing bone microarchitecture to the Discussion section (paragraph 2). We emphasized the value of HR-pQCT and mentioned the results of the most significant studies with the use of this method in patients with diabetes.
Conclusion: 1. Again, authors have included factors not significant on multivariate analysis in the conclusions.	We revised the Conclusion as recommended.
Reviewer 3	
The background of the study needs further	Thank you for your suggestion. We cited

elaboration including a brief mention about pathophysiology. The meta-analysis by Ho-Pham (10.1007/s00198-019-05053-z) assessed the association between trabecular bone score and type 2 diabetes. A comparison of the study with previous ones will be prudent and a mention of whether any unique aspects are being addressed in the current research can be considered. Some specific comments related to the manuscript are listed below.	the meta-analysis by Ho-Pham et al. in the Introduction section. Comparison of the results of our study with the results of previous works in this area, as well as pathophysiological aspects, are presented mainly in the Discussion.
1. Introduction section - "Recent data from the Continuous National Health and Nutrition Examination Survey (NHANES) indicate an increasing prevalence of osteoporosis and osteopenia in the US among T2D patients and non-diabetic subjects aged 40 years and older". - Please clarify. Did the data show increase in osteoporosis trend among non-diabetic subjects above 40 years.	Thank you for your comment. We rechecked the source and rephrased the sentence as follow: Recent data from the Continuous National Health and Nutrition Examination Survey (NHANES) indicate an increasing prevalence of osteoporosis and osteopenia in the US among T2D patients.
2. Introduction section - "In addition, the TBS decrease in subjects with pre-diabetes was demonstrated." Reframe and elaborate.	We have changed this statement and moved it to the Discussion. In addition, we have included a link to the study by Holloway et al. (2018) demonstrating no difference in TBS values between subjects with normoglycaemia and impaired fasting glucose.
3. Methodology section - The cut off for TBS was taken as 1.31 The more widely used cut offs are as follows TBS > 1.350 is considered to be normal; TBS between 1.200 and 1.350 is considered to be consistent with partially degraded microarchitecture; and TBS <1.200 defines degraded microarchitecture Silva BC, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, McCloskey EV, Kanis JA, Bilezikian JP. Trabecular bone score: a noninvasive analytical method based upon the DXA image. Journal of Bone and Mineral Research. 2014 Mar;29(3):518-30. Please clarify in the discuss section.	Thank you for your comment. Indeed, the classification of TBS by an international working group has proposed 1.35 as a cut-off point. However, a subsequent meta-analysis of the association of TBS with fracture risk (McCloskey EV et al., 2016 DOI: 10.1002/jbmr.2734) suggested that the cut-off point should be slightly lower (1.31). Considering that fractures are the most important aspect of osteoprosis from a clinical point of view, we consider this cut-off to be more reasonable. In our sample of patients, choosing a cut-off point of 1.35 would result in reclassification of only 4 cases, which would not significantly affect the results. Since there are discrepancies in the choice of the TBS cut-off, we considered it appropriate to expand the

	discussion of this issue in the article (paragraph 3 in the Discussion section).
4. Methodology section - Was country specific FRAX calculator used?	Thank you for your comment. Yes, it was. We included a clarification in the text. We have clarified the description of the method in the revised version of the manuscript.
5. Methodology section - The BMI ranged from 19.1 to 50.2 kg/m2 (median 33.6 kg/m2). Increasing soft-tissue thickness can artifactually decrease TBS values due to degradation in DXA image quality. The manufacturers recommend including patients in the BMI range of 15-37 kg/m2. Could this be a potential confounder at extremes of BMI >37 kg/m2? Does the TBS iNsight software (version 3.0.2.0, GE, USA) correct for extremes of BMI?	Thank you for raising this important issue. As applied TBS iNsight software (version 3.0.2.0, GE, USA) does not correct for extremes of BMI, the inclusion of subjects with BMI >37 kg/m2 is a limitation of our study. We include this information in the appropriate section of the manuscript.
6. Methodology section - Six women with TBS >1.31 and 14 women with TBS <1.31 had at least one fracture in their medical history ($\chi 2=5.64$, $p=0.02$). In these groups, low-energy fractures were documented in 2 and 9 women respectively Were the fractures documented by X-Ray? At what sites where these fractures found? Were lumbar vertebrae with fractures excluded from TBS measurement?	Thank you for your comment. Information about fractures was collected by interviewing the patients. Fractures of the spine were confirmed by X-ray. Vertebrae with compression fractures were excluded from the TBS measurement. The information of fractures was added into the Results and Table 1.
7. Methodology section - Were patients on pioglitazone/rosiglitazone excluded from the analysis?	Yes, we considered treatment with pioglitazone/rosiglitazone as exclusion criterion. The list of exclusion criteria was updated.
8. Results - Page 10 - In a model of multivariate stepwise regression .. correct grammar	It was corrected.
9. Discussion - HBA1C was not identified as a risk factor for low TBS scores which is in contrast to the established fact that hyperglycemia adversely affects bone health. What could be the possible explanation?	Thank you for bringing up this important issue. Most of the patients in our study had long-term diabetes and non-target glycemic control on combined antidiabetic therapy. In addition, only a single HbA1c value was included in the analysis. These factors could mask the effect of hyperglycemia on TBS. We included this explanation in the Discussion section. We have also included data from other studies demonstrating the relationship between glycemic control and TBS in the revised

	version of the manuscript.

The changes in the text of the manuscript are highlighted in green.
We are hopeful that the changes have been made based on the reviewer`s comments improved the content of our manuscript and further increased the scientific value. Thank you very much for considering our work.

Yours sincerely,
Prof. V. Klimontov