Author’s response to reviews

Title: Vitamin D, parathyroid hormone and metabolic syndrome - the PORMETS study

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Author’s response to reviews:

Rebuttal letter – response to each point raised by the Academic Editor and Reviewers.

Editor Comments:

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Reviewer reports:

Rachel Crowley (Reviewer 1):

The authors describe a statistical exploration of the relationship between 25OHD and PTH with elements of the metabolic syndrome in a subgroup of a Portuguese study cohort.

Comment #1

Methods:

1) Definition of vitamin D status using Endocrine Society guidelines has defined more than 99% of the study participants as being in a disease state, therefore the conclusions regarding the metabolic syndrome may be difficult to interpret
2) The authors should discuss why they used Endocrine Society rather than IOM guidelines

3) Use of the Endocrine Society guidelines may explain why PTH was 'blunted' - some of these patients had adequate vitamin D to meet their requirements.

4) The authors should comment on the health of the subgroup - were they attending the health centres while unwell or were they attending routine study appointments? Were they taking supplementation with calcium / vitamin D, on any antiresorptives / HRT, taking proton pump inhibitors or diuretics?

Response to comment #1

Thank you for the constructive comments on how to improve the readability of the manuscript.

1) Globally, there is a general consensus that blood 25(OH)D levels below 25-30 nmol/L (or 10-12 ng/mL) qualify as ‘deficient’ (Spiro & Buttriss, 2014), but beyond this there is currently no standard definition or agreement as to ‘optimal’ 25(OH)D levels.

The US Institute of Medicine recommendations for vitamin D (IOM, 2011), based on a review of the evidence stated: persons are at risk of deficiency at serum 25OHD levels of below 30 nmol/L (12 ng/mL); some, but not all, persons are potentially at risk for inadequacy at serum 25OHD levels from 30 up to 50 nmol/L (12 to < 20 ng/mL); practically all persons are sufficient at levels of 50 nmol/L (20 ng/mL) and above; serum concentrations of 25OHD above 75 nmol/L (30 ng/mL) are not associated with increased benefit.

The Endocrine Society Task Force (Holick, 2011), disputed IOM recommendations and published a clinical practice guideline that defined vitamin D deficiency as a serum 25(OH)D <50 nmol/L (20 ng/mL), and argued that serum 25(OH)D levels should be greater than or equal to 75 nmol/L (30 ng/mL) to maximise the effect of vitamin D on calcium, bone and muscle metabolism. These recommendations were adopted in the practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe, including Poland, Hungary, Belarus, Estonia, Czech Republic and Ukraine (Pludowski et al. 2013). However, the strength of evidence to support the need to aim for substantially higher serum 25(OH)D concentrations of >75 nmol/L (30 ng/mL) is currently unclear and most scientific societies did not adopt these values.

2) Nevertheless, and taking into account the comments of the reviewer, the Endocrine Society classification, has been replaced by the more conservative definition of the Institute of
Medicine (Methods section, lines 167-173, page 8). The results regarding the metabolic syndrome were not affected by this modification, as it can be observed in table 5, as the variable “25(OH)D serum levels” was treated as a continuous variable. In addition, the sample size is large and the variable “25(OH)D serum levels” is approximately normally distributed. Information on PTH and vitamin D variables were included in the legend of Tables 4 (line 649, page 30) and 5 (lines 675-676, page 32) to clarify the type of variables considered.

3) In fact, the authors do not consider that the use of the Endocrine Society cut-off points may explain the “blunted” PTH response. A “blunted PTH response” was defined as a PTH level within the reference range in the presence of 25(OH)D ★ 30 nmol/L/12 ng/mL (Methods section, lines 171-173, page 8). According to this definition, only participants with vitamin D values below 12 ng / mL were considered. A “blunted PTH response” was present in 89.2% of the 185 participants, with serum 25(OH)D levels of less than or equal to 12 ng/mL (Results section, lines 216-217, page 10). A discussion regarding this subject was included in the discussion section (lines 281-284, pages 12-13), according to the reviewer comment.

4) As stated in the Methods section: “In each center, participants were randomly selected from the general practitioners’ patient lists, and 120 participants were evaluated under an inclusion criterion of 18 years of age or older” (Methods section, lines 107-109, page 6). According to Portuguese legislation, all citizens have the right to be enrolled in a health care center. It was taking into account this fact that it was decided to select the participants from the lists of health centers. Given that, participants were randomly selected from the general practitioners’ patient lists (Methods section, lines 107-108, page 6), participants specifically went to the health center to participate in this study. Thus, the participants did not went to the health center because they were sick or because they had a routine appointment. According to the reviewer's comment the text has been amended to clarify this point (Methods section, lines 109-110, page 6).

A structured questionnaire was administered to collect information on personal medical histories and socio-demographic and behavioral characteristics. About 70% of the participants were on chronic medication, and probably some participants were taking drugs that interfered with phosphorus-calcium metabolism. Medication intake may have contributed to an underestimation of the prevalence of hypovitaminosis D.
Comment #2

1) Table 4 - are the associations point-biserial here?

Response to comment #2

Thank you for the relevant comment. By mistake the sentence “Pearson´s correlations were used for continuous variables, and Point-biserial correlations were used to measure the strength and direction of the association between continuous variables and dichotomous variables” (Methods section, lines 185-187, page 9 of the original manuscript) was not removed in our final review of the manuscript. For that we do apologize. In fact, we do not present Pearson`s and Point-biserial correlations. As such the sentence was removed from the statistical analysis section.

Table 4 (page 30) shows the associations of PTH and 25(OH)D with socio-demographic, anthropometric, clinical and analytical characteristics. Multiple linear regression models, with the PTH and 25(OH)D levels as dependent variables, were used to calculate the regression coefficients and their respective 95% confidence intervals (95% CI) for several independent variables. The final model was adjusted for age and sex. This information is in the statistical analysis section.

Comment #3

2) Table 5 please explain the use of odds ratio for this table. Do the authors mean odds of outcome meeting component of MetS? eg trigs >150mg/dl yes/no?

Response to comment #3

Table 5 (page 32) shows the associations of PTH and 25(OH)D serum levels with MetS and its components. Unconditional logistic regression models with metabolic syndrome and its individual components as dependent variables were computed, and the odds ratios (OR) and their respective 95% CI were estimated for PTH and 25(OH)D serum levels, after adjustments for confounding variables.

In this table, the calculated ORs represent the odds of an outcome (metabolic syndrome or its individual components) occurring for an increased unit of PTH or 25(OH)D serum levels. In other words, the exponential function of the regression coefficient (e \( \beta \)) is the odds ratio associated with a one-unit increase in the exposure (PTH or 25(OH)D serum levels).

In our study PTH serum levels presented a positive crude association with metabolic syndrome, waist circumference and blood pressure components; after adjustment for confounding variables (age and sex) only the waist component maintained a significant association. On the other hand, 25(OH)D serum levels presented a negative crude association with the metabolic syndrome and
its blood pressure and triglycerides components. After adjustment for confounding variables, only blood pressure and triglycerides components maintained the association.

The authors decided to modify Table 5 and present for each independent variable the ORs with their respective 95% CI, according to 2 models: model 1 (adjusted for age and sex) and model 2 (adjusted for age, gender and BMI).

Comment #4

Discussion

As a general comment much of the discussion is repetition of the results, followed by a list of potential explanations for findings that could either represent significant associations or limitations of the study. More explanation of the most likely explanation would be more useful for the reader. Because of the dissociation of Vit D and PTH levels, their associations are considered separately - this is confusing and suggests to me that the associations are less likely to be clinically meaningful.

Response to comment #4

The authors tried to summarise key results, with reference to study objectives, and to discuss limitations of the study, taking into account sources of potential bias or imprecision. The authors also tried to give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. According to the reviewer’ comment, the authors made some changes in the “Discussion” session to avoid redundancy between the “Results” and the “Discussion section”.

The goal of our study was to evaluate the prevalence of hypovitaminosis D and its determinants as well as PTH serum levels determinants and associations of the 25(OH)D and PTH serum levels with MetS and its individual components in a sample of the Portuguese mainland population (Introduction section, lines 95-98, pages 4-5). According to this, we evaluated some possible determinants of the 25(OH)D levels, including PTH serum levels. As already mentioned, we did not find an association of PTH with 25(OH)D serum levels. The authors attempted to delve deeper into the subject, characterizing participants with a “blunted PTH response”. Although the analysis of the determinants of serum PTH levels is not clearly included in the objectives of the study, the authors decided to include these results. Because of this, we changed the sentence referring to the objectives of the study in the Abstract section (lines 31-34, page 2) and Introduction section (lines 95-98, pages 4-5).

The lack of association between 25(OH)D and PTH does not impact on the associations found for each of them. The analysis of possible associations for each of the variables [serum levels of PTH and 25(OH)D] was carried out with the aim of detecting variables with possible
interference in the associations of serum levels of PTH and 25 (OH) D with metabolic syndrome and its components.

Comment #5

Line 383-4 does not contribute to the conclusion.

Response to comment #5

According to the reviewer’s suggestion, the phrase “The absence of an association between PTH and 25(OH)D can be partly explained by the attenuated response of PTH to hypovitaminosis D” (Conclusion section, lines 383-384, page 17 of the original manuscript) was removed.

Comment #6

The authors should re-formulate the final sentence - a finding of some associations within a cohort all of whom have low 25OHD by Endocrine Society guidelines is not evidence that supplementation of vitamin D will reverse the findings / associations.

Response to comment #6

As already stated, and taking into account the comments of the reviewer, the Endocrine Society classification has been replaced by the more conservative definition of the Institute of Medicine. According to this, the final sentence of the conclusion was modified.

Zaki Hassan-Smith (Reviewer 2):

The authors set out to characterize relationships between Vitamin D/PTH and components of the metabolic syndrome and to assess their prevalences in a Portuguese cohort. This was a sub-study of the PORMETS study (n=500 of 4096 in total study). There was a high prevalence of vitamin D deficiency. Statistically significant associations were seen between vitamin D status/PTH and metabolic syndrome markers. Notably serum PTH vs waist circumference; and serum 25OHD vs MetS, BP, TGs had negative associations. There was a high prevalence of vitamin D deficiency in the cohort.
Comment #1

1. This is a potentially useful contribution to the literature - i.e. in characterizing prevalence of vitamin D deficiency in the Portuguese population and in documenting associations with components of the metabolic syndrome. I would suggest that a caveat is made to be clear that causality has not been addressed. The associations between vitamin D/PTH and markers of the metabolic syndrome could be explained by a factor such sequestration of vitamin D in adipose tissue with increasing fat mass.

Response to comment #1

We thank the reviewer for the favourable general comments and suggestions to our manuscript.

The study presented is of an analytical cross-sectional type. We analysed the associations between 25(OH)D/PTH serum levels and a health outcome (metabolic syndrome or its components). This type of study is limited in its ability to draw valid conclusions about possible causality because the presence of risk factors and outcomes are measured simultaneously. We included a sentence to make it clear that these associations do not necessarily have a causal relationship (Discussion section, lines 383-385, page 16).

The reviewer emphasized in his commentary the contribution of adipose tissue to the associations found. In fact, we found an association of PTH and vitamin D with BMI. In addition, and according to the literature, there is an important association of the metabolic syndrome and its components with several adipose measures including BMI. Taking into account the comments of the reviewer the authors decided to include in the statistical analysis the adjustment to the BMI. Changes were made in Results section and in Table 5.

We found an adjusted (for gender and age) negative association between the serum 25 (OH) D levels and MetS and its BP and triglycerides components. After additional adjustment for BMI, all associations were maintained, with the exception of the association with the metabolic syndrome; this loss of association suggests that adipose tissue may contribute to the association between vitamin D and metabolic syndrome. The sequestration of vitamin D (and possible reduction of vitamin D levels related to the volume of adipose mass) may in fact have contributed to the association found. In contrast, adipose tissue may not play an equally important role in the association found between the serum 25(OH)D levels and the BP and triglyceride components of the syndrome.

In the case of PTH, the sequestration of vitamin D (and possible reduction of vitamin D levels related to the volume of adipose mass) may not have contributed to the observed associations with the waist component. PTH serum level maintained the positive association with the waist circumference component even after adjustment for 25(OH)D serum levels (plus age and gender). According to the reviewer’s comments some additional changes were made in the Results section and in the legend of Table 5 presenting this additional result.
Comment #2

2. It may be informative also to give proportions of subjects with deficiency according to IOM statement.

Response to comment #2

Following the recommendation of the other reviewer, we have replaced the Endocrine Society definition by IOM definition.

Comment #3

3. Has consideration been made to multiple comparisons and the potential for false discovery rate? In addition many of the associations seen are weak. Comment should be made on these points.

Response do comment #3

The problem with performing multiple simultaneous hypothesis tests, is that, as the number of hypotheses increases, so does the probability of wrongly rejecting a null hypothesis because of random. The traditional solution is to reduce the threshold p-value that is used to determine what we call a significant difference. The most renowned method for reducing the threshold of significance is the Bonferroni- adjusted p value. The use of this conservative methodology reduces very substantially the false discovery rate. However, type 2 error may increase (do not consider a significant association to be significant).

By mistake the sentence “Pearson’s correlations were used for continuous variables, and Point-biserial correlations were used to measure the strength and direction of the association between continuous variables and dichotomous variables” (Methods section, lines 185-187, page 9 of the first submission manuscript) was not removed in our final review of the manuscript. For that we do apologize. In fact, multiple linear regression models, with the PTH and 25(OH) D levels as dependent variables, were used to calculate the regression coefficients and their respective 95% confidence intervals (95% CI) for several variables and unconditional logistic regression models with MetS, its individual components or a “blunted PTH response” as dependent variables were computed, and the odds ratios (OR) and their respective 95% CI estimated for the variable tested.

Because only 3 to 4 independent variables were considered in the logistic or linear regression models, the authors considered it reasonable not to make a correction for the multiple comparisons, thus avoiding the type 2 error. In addition, the associations found are supported by previously published evidence. Nevertheless, the authors acknowledge this in the discussion section (lines 385-388, page 16), when considering additional limitations of the study.
Regarding the reviewer comment on the weakness of the associations of PTH and 25 (OH) D with MetS and its components, we think that this may be due to the fact that the variables considered (PTH and 25(OH)D serum levels) are continuous and therefore the calculated ORs represent the odds of an outcome (metabolic syndrome or its individual components) occurring for an increased unit of PTH or 25(OH)D serum levels. This discussion was also included in the end of the discussion section of the manuscript (lines 378-382, page 16).

Comment #4

4. It would be interesting to make a brief comment on previous studies that shed light on potential mechanisms of the proposed link between vitamin D/PTH status and metabolic syndrome.

Response to comment #4

According to the reviewer’s suggestion a phrase was include about the potential mechanisms of the proposed link between vitamin D and metabolic syndrome (Discussion section, lines 342-345, page 15). Regarding the mechanisms of PTH association with adipose tissue, they are already mentioned in the text. We also included an additional phase about the potential mechanisms of the proposed link between PTH and metabolic syndrome (Discussion section, lines 348-349, page 15).