Author's response to reviews

Title: Pakistanis living in Oslo have lower serum 1,25-dihydroxyvitamin D levels but higher serum ionized calcium levels compared with ethnic Norwegians. The Oslo Health Study.

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Author's response to reviews: see over
Dear Editor,

Thank you very much for your reply regarding our submitted manuscript: “Pakistanis living in Oslo have lower serum 1,25-dihydroxyvitamin D levels but higher serum ionized calcium levels compared with ethnic Norwegians. The Oslo Health Study”, as well as the qualified viewpoints of the two reviewers.

Please find enclosed our revised manuscript, and our reply to the reviewers below.

Sincerely, on behalf of the authors

Kristin Holvik
Answers to the comments of the Reviewers

The comments are reproduced in bold font and the replies are written in normal font. The paragraphs that refer to modifications of the manuscript are given in red font.

Reviewer #1:

1. In the introduction can the authors tell us how the present paper adds to references 7 and 11? Men, wider age range, sample size

Reply: We have now added the following to the introduction on page 6:

In contrast to these older studies, the Oslo Health Study 2000-2001 is a much larger, population-based study including samples of the general population. It covers a wide age range, and it also includes men.

2. Were the Pakistanis and the ethnic Norwegians sampled at the same time of the year? Was month of collection controlled for in the analysis?

Reply: The sampling took place throughout the year, including all months except February-April and July (due to summer closed screening station). Invitations were sent in a random order, and were not stratified on ethnic background or other characteristics related ethnic background. The participants were given a tentative appointment with the possibility to attend any time within the opening hours of the screening station if the appointment was inappropriate. Of the ethnic Norwegians, 80% were invited during the summer months (May –September) and 20% during the winter months (October-April), and the corresponding figures for the Pakistanis were 79% and 21%, respectively. Introducing an additional adjustment for season of attendance did not alter the observed ethnic differences in any of the metabolites presented in Table 1.

We have now added the following in the Methods section, page 7:

The participants were invited in a random order throughout the study period regardless of ethnic background.

3. Dietary calcium is know to mediate the relationship between vitamin D and PTH, was this measured and controlled for?

Reply: Although the questionnaire included some questions concerning important calcium-containing foods in a typical Norwegian diet (dairy products), this variable has not been used in the present paper. We feel that this calcium variable may not be valid for a Pakistani diet, and as this is a large population-based study, their diet has not been evaluated in full. However, vitamin D and PTH status in the present sample has been treated in a previous paper (Meyer et al., Bone 2004, 35: 412-417), and is not the main focus of discussion for the current paper. Our main focus
of interest is to evaluate how vitamin D and PTH status influences the resulting calcium levels in serum. We cannot know whether the calcium levels result quantitatively mainly from bone resorption or intestinal calcium absorption, as PTH and 1,25-dihydroxyvitamin D influence both.

4. What impact could non-fasting have on calcitriol or PTH? i.e. calcium intake

Reply: As mentioned in the Methods section, the blood samples were non-fasting. This was due to practical reasons, as it would be very difficult to require fasting in thousands of free-living persons attending a screening station throughout the day and week. We agree that standardization of time since last meal would have been preferable when analysing these blood parameters. However, time since last meal was registered, and adjustment for this did not alter the observed age-adjusted levels of any of the metabolites presented in table 1.

We have added the following in the Methods section, page 9:

The analyses are not adjusted for time since last meal as additional analyses showed that such adjustment did not influence the levels of any of the metabolites.

We have added the following in the Discussion section, page 13:

A limitation of the study was non-fasting blood samples, which could influence s-iPTH and s-Ca$^{2+}$ levels. However, adjustment for time since last meal did not alter the observed levels of these metabolites.

5. If points 2 and 3 and 4 cannot be addressed in the methods than this limitation should be discussed

Reply: Please see above.

Reviewer #2:

The recruitment period was from May 2000 to January 2001 but it is not described further if the recruitment was equally distributed for the Pakistanis and Norwegians over this time period.

Reply: Please see our reply to point 2 raised by reviewer #1.

The authors mentioned that three subjects were excluded (Norwegians or Pakistanis?) due to primary hyperparathyroidism as defined by S-iPTH >8.5 pmol/L and serum ionized calcium >1.35 mmol/L.

Reply: The excluded subjects were two Norwegian women and one Pakistani woman. This has now been specified in the Methods section, page 8.
This level of ionized calcium of 1.35 mmol/L is rather high and does not therefore exclude some borderline primary hyperparathyroid patients with ionized calcium in the upper range and PTH also within normal limits but inappropriately high. Just a few such cases could affect the relationship between ionized calcium and PTH levels which is the main finding of this paper. The authors should therefore describe better the distribution of ionized calcium and PTH level within the study group to exclude any possible difference between the study groups in this respect. This is only partly done by Figure 1.

Reply: We have now provided the ranges of the metabolites in table 1.

The blood sample collected from each participant on the day of attendance was a non-fasting one which might be a limiting factor in studying the relationship between ionized calcium and PTH as post prandial fluctuations in ionized calcium are likely to occur to some extent followed by PTH changes. The authors might therefore mention this in the discussion as a possible limitation.

As the blood samples were not collected fasting and the groups might have attended at different hours after meals, this might affect the results and the authors should address this possible problem in the design of the study.

Reply: We agree that fasting would be preferable when studying these metabolites, but for practical reasons in a large population-based study, non-fasting was chosen. We have added the following in the Discussion section, page 13:

A limitation of the study was non-fasting blood samples, which could influence PTH and Ca\textsuperscript{2+} levels. However, adjustment for time since last meal did not alter the observed levels of these metabolites.

Also, please see our reply to point 4 raised by reviewer #1.

As mentioned previously it might be useful to have the range of the variables in Table 1.

Reply: This has now been done.