Author’s response to reviews

Title: Cortical and trabecular bone are equally affected in rats with renal failure and secondary hyperparathyroidism

Authors:
Nikita Bajwa (nikita.bajwa@va.gov)
Cheryl Sanchez (csanchezkazi@llu.edu)
Richard Lindsey (richard.lindsey@va.gov)
Heather Watt (heather.watt@va.gov)
Subburaman Mohan (subburaman.mohan@va.gov)

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Author’s response to reviews:

October 24, 2017

Dr. Pascal Houillier
Associate Editor
BMC Nephrology

Re: Submission of revised manuscript (BNEP-D-17-00299)

Dear Dr. Houillier:

Thank you for your email dated October 2, 2017 and the reviewer comments for our manuscript entitled, “Cortical and trabecular bone are equally affected in rats with renal failure and secondary hyperparathyroidism “.

We have revised the manuscript by responding to the comments of the reviewers and editorial remarks as indicated in the responses to reviewer comments. We hope that the revised manuscript is now acceptable for publication in BMC Nephrology.

Please let me know if you need any further information.

Thank you for consideration of our paper.
With best wishes and regards,

Sincerely,
RESPONSES TO EDITOR AND REVIEWER COMMENTS

We thank the editor and reviewers for the careful review of our manuscript and for the positive constructive comments. We very much appreciate all your time and efforts in improving the quality of this paper. We have revised the manuscript and addressed all the comments as below.

REVIWER #1 (Dr. Ferruh Artunc)

Specific Comments:

Comment 1) A major point of criticism is that the authors don’t report the number of performed individual experiments (n) at any place in the text or the illustrations. Without reporting the number of individual experiments a publication wouldn’t be possible. In case of small n maybe it would be better to show data as scatter graphs or box plots instead of bar graphs.

Response: We apologize for not indicating number of animals per group for different measurements. We have now included the number of animals per analysis in figure legends. The number of animals chosen per experimental group (10-12) was based on power analysis and similar to what has been used in the past for similar types of experiments.

Comment 2) Figure 1A shows a point of taking blood on day twelve after starting nephrectomy. Results from these values were never mentioned neither in the text nor in the figures. If these samples exist, please show the measured values since they may contribute to the understanding of the sequence of observed changes.

Response: We apologize for this error on the timeline. It was a mistake and we have removed it from revised Figure 1A.

Comment 3) Please show weight gain and length gain as relative values (fig. 1B).
Response: As per this comment, we have made the changes in revised Figure 1B.

Comment 4) Legend to figure 1: * Significant at p<.05 versus Nx-Phos and control animals. ^Significant at p<.05 versus Nx-Phos and Nx-Control animals.

Response: We thank the reviewer for this comment and have clarified the significance values in the legend to Figure 1.

Comment 5) Please reorder the parts of figures 4-6 according to the order in the text.

Response: We apologize for this error and have revised the figure numbers per their order in the manuscript.

Comment 6) Figure 7 C uses a symbol (#) not described in the legend.

Response: We believe the reviewer was referring to Figure 6D. We apologize for the error and have revised the legend for Figure 6D.

7) Please report r, r² and p in the legend or figure at figure 7.

Response: We have included the r² and p value in the legend for Figure 7.

Comment 8) The last sentence of the discussion should end with the word rats instead of mice.

Response: We apologize for this error and have revised the text in the discussion.

Comment 9) Use the term used in the text for the group-headings in table 1 and 2.

Response: We thank the reviewer for this suggestion. We have revised the headings in Table 1 and Table 2.

Comment 10) Please show representative bone sections and micro-CT scans.

Response: We thank the reviewer for this suggestion. We have included representative micro-CT bone sections in revised Figure 5A.

Comment 11) Could something be said about the extent of movement of the individual animals? It is conceivable that reduced muscle action as it is to be expected in CKD with developing uremia may lead to changes in bone structure (e.g. Hart NH et al., Mechanical basis of bone strength: influence of bone material, bone structure and muscle action. J Musculoskelet Neuronal Interact., 2017).

Response: We agree with the reviewer suggestion regarding evaluating muscle-bone connection. We did not detect any changes in lean body mass (measure of muscle mass) as measured with DXA at any time point in the study, suggesting that movement of the animals were not impaired.
Comment 12) The hyperphosphatemia model is somehow artificial since CKD patients would not be advised to increase phosphorus load. Please discuss the expected effects of low phosphate diet/phosphate binder as main therapeutic instruments used in CKD-MBD as shown in the paper of Bohnert et al. (Impact of phosphorus restriction and vitamin D-substitution on secondary hyperparathyroidism in a proteinuric mouse model. Kidney Blood Press Res., 2015).

Response:
We agree with the reviewer that it would be ideal to lower dietary phosphorus to prevent the development of hyperphosphatemia and worsening secondary hyperparathyroidism. Patients especially children with chronic kidney disease oftentimes do not follow low phosphorus intake since the food is unpalatable, hence the use of phosphate binding agents are frequently used. Our experiment is to reflect the skeletal effects of hyperphosphatemia and severe secondary hyperparathyroidism in kidney disease. We did not use dietary phosphorus restriction in the Nephrectomized Control group since we wanted to compare the Nephrectomized Control group with mild increase in PTH to severe secondary hyperparathyroidism.

Comment 13) What’s the explanation for the great difference (more than doubling) between day 19 and day 26 in creatinine values of the control group?

Response:
Creatinine is a reflection of muscle mass so the increase in serum creatinine may be related to the increase in weight in this group of growing rats.

Comment 14) It would be interesting to see 25 and 1,25 Vitamin D concentrations.

Response: We thank the reviewer for this suggestion. Unfortunately, we are unable to do these measurements as we don’t have adequate amount of serum left for many of the animals.

Pascal Houillier (Reviewer 2): The authors made an attempt to uncover the mechanisms linking phosphate retention and bone disorders in early CKD.

Major concerns:

Comment 1) The model is acute rather than chronic kidney disease

Response:
Although the length of the study is only 4 weeks, in the lifetime of the growing rat, the skeletal changes reflect histologic findings in chronic kidney disease as shown in multiple published studies.

Comment 2) The authors have been studying young rats with a high velocity of growth. Therefore, it is extremely difficult to distinguish the consequences of phosphate retention and those of altered growth due to impaired renal function
Response:

Our previous studies (see reference below) have shown that growing nephrectomized rats on regular phosphorus diet showed normal growth plate architecture with mild elevations of serum PTH and serum phosphorus comparable to growing rats with normal renal function. The growth plate architecture in phosphorus loaded rats with severe secondary hyperparathyroidism showed distortion of the growth plate architecture. It is difficult to delineate whether the higher phosphorus level or the higher PTH levels are responsible for the changes in the growth plate.


Comment 3) The authors claim being interested by the events occurring at an early stage of CKD. However, they performed 5/6 Nx and have not quantified renal function better than by creatinine measurement. A reliable assessment of renal function is required to show that experiments have been carried-out at early stages of CKD

Response: We thank the reviewer for this comment. The 5/6 nephrectomy model is a well-established model for chronic kidney failure which is more predictable than using the adenine diet model. Although the best measure of kidney function is to measure nuclear GFR, majority of studies use serum creatinine to follow renal function in majority of experimental studies (Ota et al., 2017; Fernandes-Charpiot et al., 2016). We have previously used this model to evaluate the adverse effects of renal failure and secondary hyperparathyroidism in young growing rats with consistency (Sanchez & He, 2003; Sanchez & He, 2004; Sanchez et al., 2004; Sanchez & He, 2005; Hooper et al., 2007).

Comment 4) The plots on Fig.7 are highly scattered and not quite convincing. In addition, they do not provide any insight into the molecular mechanisms going on as no attempt to interfere with any of the potential pathways has been made

Response: We thank the author for the comment. We have addressed the significance and interpretation of the scatter dot plots in the revised discussion section.

Miscellaneous:

Comment 1) The number of rats in each group is unknown

Response: We thank the author for this comment. We have added the number of rats in each group in the revised figure legends.

Comment 2) the values of Pi (Fig.6) must be checked (? What does this mean?)

Response:
The serum phosphorus in nephrectomized control and intact control animals were comparable as shown in our previous studies (see reference below). It is only in the phosphorus loaded animals that serum phosphorus increased. We did not measure TMP:GFR which will be able to compare whether the remaining nephrons are excreting more phosphorus compared to rats with normal renal function.