Author’s response to reviews

Title: Chemical and biomechanical characterization of hyperhomocysteinemic bone disease in a novel animal model.

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PDF covering letter
We thank both reviewers for their careful read of our manuscript and helpful comments, which we address as follows.

**Reviewer Alana Majors**

**Compulsory revisions**

1) Table 3 typo – Corrected
2) page 15 and page 16 – We have previously published ultrastructural evidence of a collagen defect (*Scanning Microscop 1990;4:667*), but the citation was mistakenly omitted. Because the explicit reference to cross-linking on page 15 may be misinterpreted as a reference to biochemical data, we have modified the sentence to read “associated alterations in collagen morphology” (p.16; l.5) and added the citation. The citation is also attached to the clause in the parallel sentence now on p.17 (l.4).

**Discretionary revisions**

1) Although the chick model is not new, several aspects of our version are. In comparison to Hill et al. (*J Nutr 2002;132:2143*), for example, ours is based on a higher intake over a longer period of time, a version we think is more likely to mimic the effects of the marked hyperhomocysteinemia seen in CBS-deficiency homocystinuria in humans. However, it is simple enough to remove the adjective, as we have done in our revision.
2) Prof. Majors makes a very valid point that there are different forms of homocystinuria in human and only one appears to be clearly associated with skeletal abnormalities. We have added the adjective ‘classical’ at the beginning of the first sentence of the abstract to eliminate ambiguity.
3) The question of comparative homocysteine biochemistry is a complex one, as there appear to be several factors at work. For total plasma homocysteine, at least, the major difference between rodents and humans is the presence of a free cysteine sulphydryl on circulating albumin, and non-protein bound fractions (perhaps the physiological ones?) are not so different. Similar data are lacking for avian species, and we agree that this is a non-trivial point. We would point out that the control-to-experimental ratio in the chick is within the range of ratios between human normal values and values in CBS-deficiency human homocystinurics. However, we hesitate to add to the manuscript, and we have not addressed this point in the revised manuscript.
4) Typo on p. 16 corrected by deletion of “with”.
5) Although there are liver changes in human CBS-deficiency homocystinuria, the presence of clinically evident liver disease is rare. To clarify this point, we have added the adjective ‘marked’, allowing us the point that the mouse model is different, in that clinically evident liver disease is more typical of that model. This bears directly on the bone changes in animal models, since clinically evident (or severe) liver disease in humans (and probably in mice) may itself cause bone
changes (so-called ‘hepatic osteodystrophy’), while milder forms of fatty liver do not.

6) The reviewer is correct to question the 8-fold ratio for CBS deficiency. In fact, the ratio varies widely, both for the total Hcy and the free fractions. We have generalized this comment by suggesting the ratio is typically greater than 5-fold., citing a recent informative paper on this subject (p.16; 6 lines from bottom).

7) Table 1— CaHPO4 typo corrected.

8) We noted the study of MacLean et al. with interest, confirming as it does the speculations of Pyeritz and others which we cited at the top of page 14 in our discussion. We have added a new sentence, at the end of that paragraph (p.14; ll.9-11 in the revision).

**Reviewer Prof Scott Miller**

Discretionary revisions

1) Done
2) Done
3) We have modified the legend of Figure 3 to incorporate this point.
4) As we state in the methods, the bone for mineral content was derived from the fracture site, which was typically the mid-diaphysis. Thus, we could only speculate as to the mineral composition of the metaphyseal regions. Unpublished histopathologic observations suggest that the defect(s) are most prominent in trabecular bone, but it would be difficult at this stage to exclude a subtle cortical component. Our studies in this area are ongoing. To be more specific on this point, however, we have indicated in the legend to Figure 3 that the defects we address here are in the trabecular bone.

5) Done.