Reviewer's report

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

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Reviewer: Alana K Majors

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Accept after discretionary revisions

This study investigates the effects of hyperhomocysteinemia on the biochemical, biomechanical, and morphologic features of bone in a chick model. The authors report significant differences in tibial length, strength, mineral composition and radiological features in chicks with hyperhomocysteinemia compared to control chicks. This may be a useful model for examining the effects of homocysteine on connective tissue.

Compulory Revisions

1) Table 3 - The S.D. and the asterisk (for significance) are missing for the carbonate/ phosphate ratio in the experimental animals.

2) Page 15, final sentence - "....and associated alterations in collagen cross-linking in this model...." Also, page 16, in the Conclusions "...despite the collagen molecular defect..."- No evidence is presented to demonstrate a defect in collagen cross-linking in these animals.

Discretionary Revisions

1) The title states this is a novel animal model, but fast growing chicks have been used previously in numerous investigations of connective tissue, many of which are included in the references. More specifically, they have been employed in studies investigating the effects of homocysteine on extracellular matrix (Hill et al., J Nutr, 2002).

2) Abstract - first line- "Homocystinuria is an autosomal recessive disorder caused by cystathionine beta-synthase deficiency and characterized by distinctive alterations of bone growth and skeletal development." CBS deficiency is only one cause of homocystinuria, there are many others (as described in reference 1). Of the many causes of homocystinuria, only homocystinuria due to CBS deficiency is associated with skeletal alterations (Skovby, In Connective Tissue and Its Heritable
3) It should probably be noted that homocysteine metabolism appears to be regulated quite differently in broiler chicks, as evidenced by their extraordinarily high basal levels of fasting plasma homocysteine - many fold higher than other species.

4) Page 16, second paragraph, line 3, the word "with" should be deleted.

5) Page 16, second paragraph - the authors state that liver disease is not typical of human CBS deficiency. Many post-mortem examinations have shown that fatty liver is a feature of CBS deficiency. References 1 and 6 also mention liver disturbances found in patients with CBS deficiency.

6) Page 16, second paragraph - the authors state "...compared to the 8-fold increase that is typical of human CBS deficiency...". This portion of the statement needs to be referenced. Homocysteine concentrations are often elevated much, much more than 8 fold in CBS deficiency. How was this number obtained?

7) Table 1 - The space in "Ca HPO4" should be removed.

8) Maclean et al. (2002, Human Mutation) described a group of patients with CBS deficiency and very high plasma homocysteine levels that do not have connective tissue defects. They suggest that elevated homocysteine alone is not responsible for the connective tissue abnormalities. Rather, a deficiency of cysteine may be responsible. Patients with homocystinuria due to other causes also do not have connective tissues problems, perhaps because they do not have low plasma cysteine. Many of the indices of bone metabolism measured in this report were normal. A combination of high homocysteine and low plasma cysteine may be required to obtain more severe connective tissue abnormalities. In future studies, it would be interesting to examine bones from animals that are hyperhomocysteinemic and hypocysteinemic.

Competing interests:

None declared.