Reviewer's report

Title: Hind limb unloading of mice modulates gene expression at the protein and mRNA level in mesenchymal bone cells

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Reviewer: David Monroe

Reviewer's report:

The manuscript entitled “Hind limb unloading of mice modulates gene expression at the protein and mRNA level in mesenchymal bone cells” by Visigalli et al. involves the study of the effects of hind-limb unloading (HU) on expression of a variety of genes during HU-induced bone loss. The authors investigate this at both the mRNA level (using in situ hybridization, ISH) and the protein level (using histoimmunochemistry, IHC). They demonstrate that both osteogenic and matrix-related markers are downregulated following HU and that these phenomena are reversible upon hind-limb reloading. The microscopic pictures (Figs 2-3) are of excellent quality and overall this manuscript has some interesting data. I have a few comments for the authors to consider and revise:

Minor essential revisions:
1) There are a number of English grammar and pluralization problems throughout the manuscript, which need to be corrected.

2) Figures 2 and 3 are unnecessarily complicated and are difficult to examine in the presented form. I would suggest that these micrographs be split up into separate figures based on the functional groupings defined by the authors in the Methods section on the Abstract (e.g. osteogenesis, matrix formation, remodeling, bone homeostasis).

3) The labels on Figures 2 and 3 are confusing and do not provide sufficient information to properly understand and follow the data. Use of both ‘A’ and ‘a’ are especially distracting. I would suggest use of Roman Numerals to differentiate between these labels. It would also be helpful if the columns representing various bone sections (e.g. growth plate, cortical bone, trabecular bone, bone marrow) are labeled on the figure itself. These authors rely on the Figure legends alone for understanding, which are unfortunately confusing and lack sufficient information.

4) The data and discussion of the angiogenesis-related gene, VEGF-A, do not contribute to the manuscript. There was no observed difference in VEGF-A expression between control and HU mice. The authors speculate in the Discussion that the lack of ability to detect any difference in VEGF-A expression may be due to the probe’s inability to discriminate between splice variants, making it entirely unclear whether the lack of a difference is biological or technical. Therefore, I would suggest removing any reference to VEGF-A from
the manuscript, unless the authors have a compelling reason to include the data.

Discretionary revisions:

1) Osteocytes are typically thought to sense changes in load in bone. Are there any changes in osteocyte-derived mRNA and protein in these animals? What about the number and quality of the osteocytes themselves? The authors may consider examining the product of the Sost gene, sclerostin, for differences between the groups. At least, the authors need to discuss any potential contribution of osteocytes to the observed phenotype and changes in gene expression.

2) What about changes in cellular apoptosis? If bone sections are still available from these mice, the authors may consider performing a TUNEL assay, or IHC for an appropriate apoptotic marker.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests