Reviewer's report

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

Version: 1 Date: 4 March 2010

Reviewer: Melissa Kacena

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

This is an interesting and comprehensive manuscript which characterizes the changes in expression of many genes/proteins with hindlimb unloading (HU) and reloading.

Major Compulsory Revisions and Discretionary Revisions

Based on my interpretation of the journal's intent I believe there are no major compulsory revisions or discretionary revisions, the revisions fall into the minor essential revisions category.

Minor Essential Revisions

1. Because of the comprehensive nature of this manuscript (at least 14 separate genes/proteins are evaluated in 4 locations within the tibia in 4 separate experimental groups) it difficult to easily digest and interpret the data. It would make the manuscript much easier to read if tables were generated. Two or three separate tables would be valuable. 1 table should be in the background/introduction section. This is a very long section as written. It is understandable why the authors did this, to make sure that the readers understand the function of the different proteins. However, this could be nicely consolidated into a table for ease, such as name, function, cells express etc. Then the results section should contain 1 or 2 additional tables depending on how they are designed. These tables would summarize the protein/gene expression findings. Having some sort of scoring system would be useful, maybe with arrows showing increases or decreases compared to control levels. This would allow for ease in understanding what changes are occurring in the system.

2. Wording is informal/awkward/incorrect in many areas and should be modified. For example, in the abstract chondro, osteo, should be chondrogenic, osteogenic. There are several similar problems throughout the text which if modified would improve the manuscript.

3. Do not capitalize the proteins in the abstract and elsewhere (e.g. osteocalcin, alkaline phosphatase, etc)
4. The text should be edited for language usage and clarity. The last sentence of the results section in the abstract is just one of the many examples where the text needs to be reworded for clarity. Another example first sentence page 3, “long term bedding” “long term bedrest” might be a better choice of words.

5. The discussion is another area that needs much work on the editing, especially page 18. Some of this is not editing so much as you are opening up potential can of worms without providing justification. I think it would be best to eliminate the last sentence of the paragraph with “transdifferentiate” as that is controversial and your data really does not speak to this. Earlier in the same paragraph the sentence “We have no suggestion to offer….peculiarities” should be modified. And, the sentences in between these, discussing adipocyte degeneration should perhaps mention marrow fat content observed, much in every micrograph.

6. With the exception of the second to last line, the conclusion is arguably the best section in terms of writing. I would formalize the writing in the second to last line and get rid of the quote. Just use recovery or some similar term.

7. Abbreviations should be made the first time they are cited.

8. The data should be consistently shown. It is confusing and unclear why 3 day data is only shown in a portion of figure 1B and nowhere else. It seems like 3 day data should either be dropped completely or included in other sections.

9. On a similar note why are day 7 data missing from figure 1B c&d? Figure 1B should also include HU14+14 micrographs. It would also be of value to include HU14+14 data in Figure 1C and 1D.

10. It would be useful for the authors to add more details to figures 2 and 3 in terms of labeling. For example identifying the rows and columns. These are very nice figures, but they contain much information, and having to go back and forth between the figure legend and the text is cumbersome. Since there are not page restrictions these could be divided into more figures to allow for the labeling.

11. Why are pictures missing in some of the composites for Figures 2 and 3. Without an explanation this is concerning as it could be thought that the results didn’t align the way the authors wanted, so the data was dropped. Those micrographs should be included or an explanation provided as to why they are missing. And why are day 7 or HU d14+14 missing from several of the groups in Figures 2&3? Again, this is troublesome when no explanation is provided.

12. A sentence on pg 15 needs to be restructured as how it reads is not accurate based on the data. This is the last sentence in the second paragraph. It states, “…did not find any discrepancy between the changes in the level of genes and proteins expression”. The first problem is that this is not a grammatically correct sentence. But the more important issue is that this is in conflict with your results for osteocalcin.

13. It is difficult for me to interpret the alkaline phosphatase data (Figure 2Aa,b)
as the authors have, perhaps this is from the micrograph displayed or from the quality of the image I see. I do not see the dramatic drop between controls and day 7 (2Aa,b) in the marrow cavity, in fact it looks higher in 2Ab to me.

**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'