Reviewer’s report

Title: Knee loading stimulates cortical bone formation in murine femurs

Version: 2 Date: 28 June 2006

Reviewer: Sundar Srinivasan

Reviewer’s report:

General

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

General Comments

The authors present a study describing the effect of knee loading upon bone formation at the femur mid-shaft. While the study is well conducted and the authors can address the minor essential revisions readily, the lack of novelty of the current paper is of major concern.

The current study essentially replicates a previous study reported by the authors where knee loading was found to enhance bone formation/area at the tibia mid-shaft. The current study is different only in that observed responses are reported at the femur mid-diaphysis (vs the tibia mid-diaphysis) and includes an additional group of animals (20 Hz group). As such, it is felt that the current study does not really extend knowledge of the field in a substantial fashion. However, given their experimental design, the authors might already possess data to address the questions below, and thereby increase relevance of their study sufficiently to warrant publication.

Does knee loading influence osteoclast dynamics at the femoral mid-diaphysis?

Given that knee loading enhances bone formation at the mid-diaphyseal site distant from point of application of loading, what is the response of bone at the epiphysis, metaphysis? Is there an inhibition/enhancement of bone adaptation at the site of loading application? And while this might not be as straightforward, do the authors have any feel as to whether knee loading has deleterious adaptive alterations upon cartilage tissue at the knee joint?

Also, given the authors (valid) point that strengthening the femur is important, how does knee loading influence the femoral neck (predominant site of aging/menopause related femoral fractures??).

Assuming that this study utilized separate groups of mice compared to their previous study (JAP, 2006), how did the current experiment influence bone formation at the tibia mid-shaft? And is the frequency dependence of bone response similar in the femur vs tibia mid-diaphysis in the current study?? And even if the mice used were the same between the two studies, citing previous data appropriately and addressing this point more explicitly (why do the femur and tibia respond optimally at different frequencies?) can enhance value of the paper.

In summary, the paper requires additional data/analysis to extend current knowledge and for it to be deemed sufficiently different from the previous paper showing essentially the same result knee loading enhances cortical bone formation and that bone response is frequency dependent. Having said this, the authors may have a novel loading modality that can enhance bone formation in the tibia AND the femur. As such, if the authors can more fully characterize bone responses at different sites along the femoral bone (particularly at the point of application of loading), such an analysis would be extremely useful in extending current understanding regarding the utility of knee loading as a noninvasive means to enhance bone structure.

Specific Comments - Discussion Section:

Para 2, Page 8: The authors briefly quote their previous study where knee loading enhanced bone formation at the tibial diaphysis. In the current study, a similar protocol enhanced bone formation at the femur mid-diaphysis although at different frequencies. Why does this happen? Is the underlying bone growth at the tibia and femur mid-shaft different? The reason for this question is that given the labeling schedule, the
detected response (in both the previous and current studies) appears to be indicative more of whether knee loading altered underlying bone growth in the 14-wk mice (as opposed to whether knee loading initiated de novo bone formation).

Para 2,3 Page 8: If knee loading enhances/alters intramedullary marrow pressure, why is it that knee loading enhances periosteal but not endocortical bone formation?? In this context, the authors suggest that (Para 2, Page 9) that their results are consistent with the tibia bending and ulna axial loading studies to name a few. However, in those studies (bending, axial loading), peak strains at the periosteal surface are substantially larger than at the endocortical surface (secondary to bending or bending induced when long bones are subject to axial compression) - hence, a lack of response at the endocortical surface (or smaller response compared to the periosteal surface) is to be expected. However, in the current study (knee loading), if IM pressure is involved, the endocortical surface should be expected to display an enhanced response compare to the periosteal surface, but no endocortical bone formation is detected here. Why is this so?

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Methods Section:
The authors have employed a labeling schedule- 2 and 6 days following the last loading. So, the response measured is in reality reflective of how knee loading altered underlying bone growth (as no knee loading was performed when the mice were injected with labels). Please clarify throughout the paper.

Results Section:
The data in Figure 4 represents a combination (average™) of data from groups exposed to varying frequencies. It is not clear why this was done as it is completely inappropriate. Table 1 already address the question of whether dynamic histomorphometry measures in loaded bones in individual groups (i.e., 5, 10,15, 20 Hz) significantly different from controls. Please omit Fig 4 as a result.

It is suggested in Para 3, Page 6 that unlike the periosteal surface, observed on the endosteal surface (data not shown). Actually, the endocortical data is present in Table 1. Please correct the statement.

Regarding the static histomorphometry data (Fig 6), please include data for marrow area along with that for cortical area and thickness. This will allow an indirect appreciation of whether knee loading alters osteoclast activity (i.e., marrow expansion?).

Discussion Section:
Para 1, Page 8: The statement Given the same loading cycles, the estimated in situ strains was < 1/10 of the predicted threshold value is quite unclear. Not sure what threshold value the authors are referring to.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field
Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests