Reviewer's report

Title: Knee loading stimulates cortical bone formation in murine femurs

Version: 2  Date: 29 June 2006

Reviewer: Clinton Rubin

This is an interesting model and manuscript confirming the sensitivity of bone to frequency, and that new bone can be formed at a site distant from a very low load. It is not immediately clear if this is the same or a different response to that observed in the tibia, reported previously (Zhang et. al., J.A.Phys, 2006), and thus what new information is provided here (other than it also stimulates bone in the femur). Further, while the authors emphasize the importance of bone strength, the material properties of the femur are never actually evaluated (something which would represent an advance over the previous paper. Additionally, that no true controls were included, no sham loading (one 0.5N load for 3 minutes), nor no baseline control data provided, it is difficult to determine if the handling of the animals somehow suppresses the overall response of bone (the anabolic response is relative to the contralateral control, but is it increasing relative to normal growth?). As importantly, given the response is periosteal and not endosteal, and that it is at a site remote from loading, there is real concern that this is evidence of Frost’s Regional Acceleratory Phenomenon; that this is not bone adaptation but rather bone repair. This is particularly pertinent as the authors state that they are essentially squeezing the bone (rather than bending it), and particularly because they are implying (last sentence of discussion) that they might try this on people, they must show that there is no damage to the cortex where the distortion is occurring, and that there is no damage to the synovium or cartilage. Points specifically to be addressed include:

1. How is this different than that reported in the JAP paper on the tibia?
2. Why not include the pressure data, as evidence that this correlates (or not) with the bone formation? Why put it in another manuscript? (or why not put this data in that manuscript?).
3. What evidence is there that the bone is strengthened? (as importantly, that the bone is not weakened?). Is increase in cortical area the key to overall bone strength?
4. What is the true strain environment at the level of the epiphysis? As the authors state, it is weaker in this region, and a full evaluation of both the strain environment, and any potential damage, must be performed.
5. What does the stimulus do to the trabecular bone beneath the loading platens? (this might also represent data beyond reporting femur vs. tibia).
6. Not only is 3 minutes of 20Hz give 4x the cycles as 5Hz, the strain rate is 4x higher. Some control should be performed to equilibrate cycle number or strain rate.
7. Some evidence that the response is a graded, as a function of time (e.g., histology of animals at 3, 6 & 9 d) would help demonstrate that this is not RAP and repair, but adaptation.
8. Some evidence that the response is not graded, as a function of the length of the bone, would help confirm that this is not repair, but instead mediated by some parameter such as fluid flow.
9. True controls, a sham loaded animal, and baseline controls are needed. This is particularly true as the variation in the control side of the loaded animals appears to be significantly different between groups (see Table 1). With that in mind, it appears as if the absolute values of the cross sectional areas, between loaded limbs as a function of frequency, is actually very similar (e.g., Figure 6a).
10. Second paragraph of discussion, I am not certain the word remarkably is warranted in terms of efficacy.
11. In that same paragraph, in discussing factors which can contribute to the observed bone formation, repair of damage should be included.
12. As no pressure data is provided, Figure 7 is speculation, and should be removed. Perhaps if the pressure data are included, it would make sense to reintroduce it.

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 limited interest

Quality of written English: Acceptable
Statistical review: No

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