Role of Receptor Activity Modifying Protein 3 in the Response of Bone to Mechanical Loading

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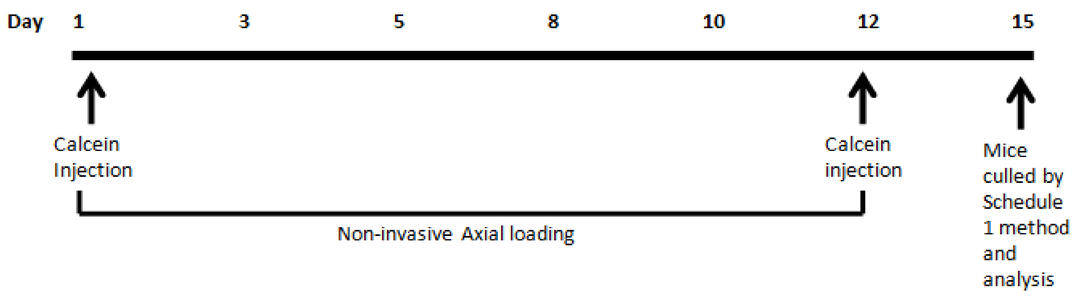
Introduction

Receptor Activity Modifying Protein 3 (RAMP3) is a single pass trans-membrane domain protein which interacts with the calcitonin receptor (CTR) and calcitonin-like receptor (CLR) in order to aid trafficking to the cell surface and to change ligand selectivity. The adaptive responses of the skeleton to loading changes the architecture and physical properties, in order to optimise strength for function. However, bone is subjected to many local and circulating osteotropic factors, many acting on G-protein coupled receptors, a family to which the CTR and CLR belong. Previous work in our laboratory has shown that RAMP3 knock-out mice have a high bone bass phenotype and a leaner body mass through to old age.

We hypothesised that as the RAMP3$^{-/-}$ mice have been shown to have a high bone mass phenotype at earlier time points, their bones would respond less to mechanical loading than wild types as they already have a skeleton which is adapted to supra-physiological loads.

Materials & Methods

Cyclical dynamic loads were applied to the left tibiae of 17 week old RAMP3$^{-/-}$ (n=8) and WT (n=7) male mice, using a trapezoidal waveform, with peak compressive loads of 13N, engendering high physiological strain magnitudes of 180,000 microstrain per second on alternate days for two weeks.



The mice were injected with calcein (100mg/kg) on day 1 and 12 for dynamic histomorphometrical analysis. Both hind limbs of the mice were analysed using microCT, reference point indentation and histomorphometry. MicroCT was performed using a SkyScan 1172 microCT scanner (Bruker-MicroCT, Belgium) at both a low resolution of the whole bone (pixel size 17.5µm) and high resolution scan of the distal femur and proximal tibia (pixel size 4.4µm). The growth rate of the bones was assessed using dynamic bone histomorphometry.

Results

The results show that both RAMP3$^{-/-}$ mice and WT bones respond well to loading (figure 4). There is no difference in the overall bone volumes, however the RAMP3$^{-/-}$ have a 66% greater bone volume/tissue volume of the new bone laid down in response to loading, than the WTs (figure 3). In areas of the bone which have had a minimal response to loading, the RAMP3$^{-/-}$ bones appear to be growing slower (figure 5).

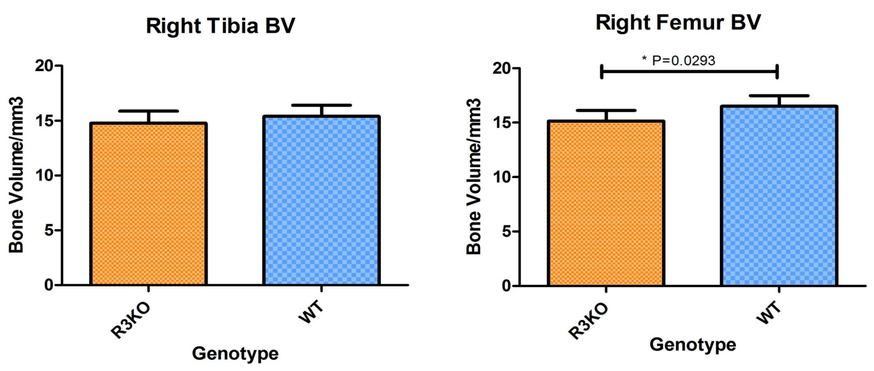


Figure 1: The bone phenotype of the RAMP3$^{-/-}$ and SvEV129 (WT) mouse strains were assessed. The WT had a 4.1% greater BV in the right tibia but there was no statistical significance between the groups (P=0.6894). The RAMP3$^{-/-}$ mice had a 8.2% lower BV in the right femur than the WT (P=0.0293)

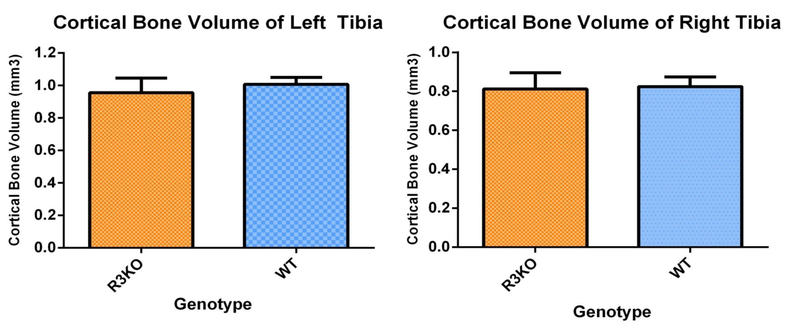


Figure 2: A comparison was made of the cortical BV of the loaded limb between the two genotypes. Overall, the WT group had a 5.1% greater cortical bone volume than the RAMP3$^{-/-}$ mice, however there was no statistically significant difference between the groups (P=0.3129).

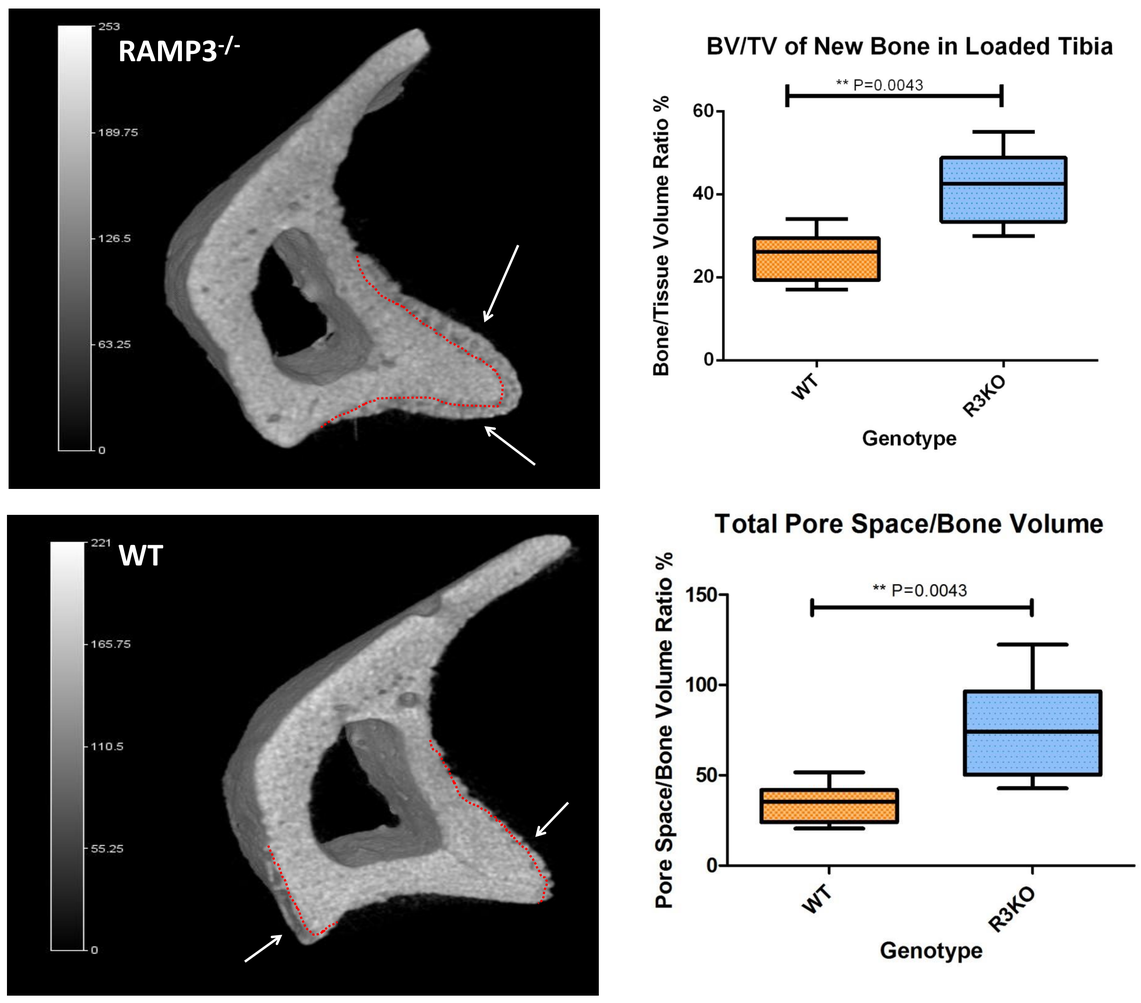


Figure 3: Comparison of 3D reconstructions of the left tibia cortical region appears to show a greater amount of new bone on the posterior surface of the RAMP3$^{-/-}$ left tibia than the WT. The new bone area also appears to be greater in the left tibia than in the right tibia in both groups. The BV/TV of the new bone area on the anterior surface shows that the RAMP3$^{-/-}$ have more new bone than the WTs (difference is 66%, P=0.0043). The area of immature bone was higher in the RAMP3$^{-/-}$ (P=0.0043).

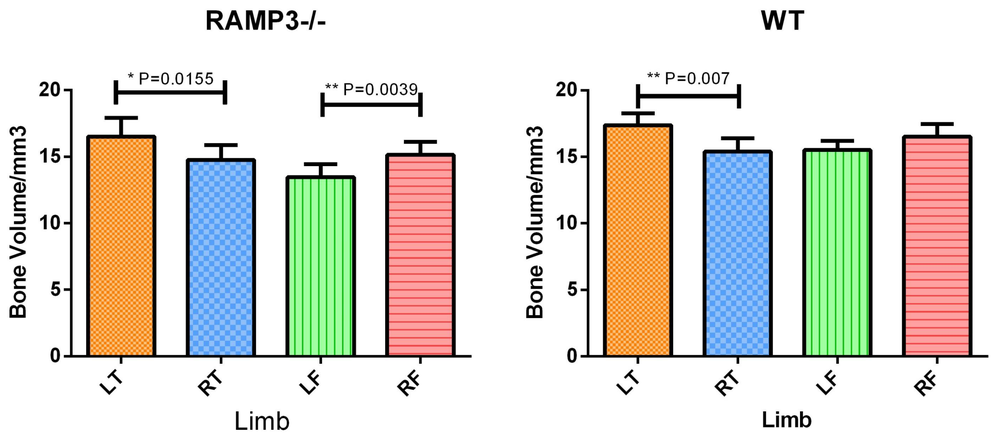


Figure 4: There was a statistically significant difference in the bone volume of the left and right tibia within each of the groups. The BV of the left tibia in the RAMP3$^{-/-}$ was 11.9% greater than the right (P=0.0155) The BV of the left tibia in the WT group was 12.9% greater than the right (P=0.007)

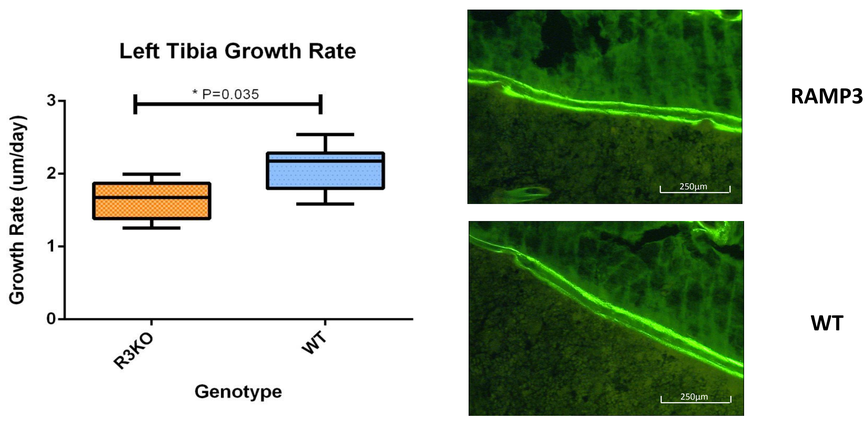


Figure 5: Dynamic histomorphometry revealed no significant difference in the double label perimeter (P=0.3129) in the left tibia across the two genotypes. However, the bone formation rate was lower in the RAMP3$^{-/-}$ mice (1.64±0.26 µm/day) compared to the WT (2.09±0.32 µm/day), P=0.035 in an area of bone in which the loading had minimal effect.

Conclusion & Future Work

This work has shown that loading induced significant changes in both WT and RAMP3KO animals with the RAMP3KO mice producing ~50% more bone than WTs in the response to loading. The basal bone formation rate in RAMP3KOs is slightly lower than WT in an area of the tibia which has shown to have little response to loading. These results are consistent with an ability of RAMP3 to exert an inhibitory effect on bone formation, not through a change in sensitivity to mechanical loading, but through a receptor mediated endocrine or paracrine response.

Future work

- Quantify the volume and distribution of new bone formed
- Assess strains using Finite Element Modelling
- Loading on 46 week old mice