4D exploration of the mechanostat: is it time to move on?

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Aims

Bone tissue adapts to mechanical forces produced by the physical activities experienced. Harold Frost inferred that the existence of a homeostatic regulatory mechanism in bone is responsible for sensing changes in the mechanical demands placed on bone and subsequently altering bone mass and architecture to better meet these new mechanical demands. He theorised that below a certain threshold of mechanical use, bone is resorbed, and is therefore rid of excess mass. Above another threshold, in which bone is exposed to greater than typical peak mechanical loads, bone formation occurs on the existing structure to increase bone strength. Thus, Frost suggested that bone tissue to have an intrinsic "mechanostat" which regulates bone functional adaptation. This mechanostat theory infers that there is simply a corresponding loss of the bone accrued, in response to this excess loading, upon the restoration of normal, habitual use yet this has never itself been tested. Until now, it has been impractical to test test this theory directly due to the need for either multiple large cohorts or the unfeasibility of the required longitudinal cohort studies. Herein, we have assessed using *in vivo* micro-CT the longitudinal load-induced bone adaptation along the length of tibia during a 2 week-long period of loading and then for a 12 week-long post loading period.

Method

The right tibia of eighteen 12-week old female C57Bl/6J mice was subjected to non-invasive *in vivo* tibial dynamic loading at 12N, 2Hz, with 10sec rest periods between cycles, 40 cycles/day, three times/week for 2 weeks. Tibiae in the right hindlimbs of 6 separate mice served as an additional non-loaded control and both loaded and control groups returned to habitual use otherwise.

Scanning of the tibia was achieved using the 1176 Skyscan (Skyscan, Kontich, Belgium) *in vivo* scanner, under anaesthetic, at 40kV, 600μA, 9μm voxel size with an exposure of 2000ms and a rotation step of 0.800 degrees. Mice were scanned at 6 time points; 3 days prior to the start of loading, as well as after week 1 and 2 of loading. Scans were repeatedat 3 days and at 6 and 12 weeks after completion of the loading regime (at 20 and 26 weeks of age, respectively) . Six mice from the original group of 18, were sacrificed at each of these latter time points. The control group had the same number of scans within the same timeframe but without any applied loading.

The slices were then reconstructed using NRecon 1.1.7.1 (Skyscan, Kontich, Belgium) and 3D registration was performed using DataViewer. Data relating to formation and resorption were obtained using CT Analyzer 1.16.9. Whole bone analysis was performed and finally, CTvox was used for 3D visualization.

Results

Our data indicate that loading leads to rapid and highly significant increases in bone cross sectional area (Fig.1) and mean cross sectional thickness (Fig. 2) within the first week of load application; these are most marked proximally and extend to encompass almost the entire tibia immediately post loading. Such increases due solely to growth in the control group were minimal and, intrigueingly, revealed that one highly localised mid-distal tibial region maintains a continuation of these increases into the immediate post-load period. Examination of the more prolonged 12 week-long post loading period, showed that the bone accrued rapidly in response to loading is lost within 6 weeks post-loading in the most proximal regions (~10-30% length). In contrast, we find that such reversal is not evident in the tibial mid-shaft region even at 12 weeks post cessation of load (~40-70% length; Figs. 1-2).

Conclusion

In conclusion, performing a longitudinal cohort study using Skyscan's *in-vivo* micro-CT, has allowed us to test Frost's mechanostat theory in 4D, with time as a variable. In accordance with the mechanostat theory, we found that much of the load-induced bone accrual confined to proximal tibia is indeed simply reversed upon removal of the original osteogenic stimulus and a return to solely habitual use. In contrast, we show no such reversal in the more distal region of bone, where the load responses continue at least for some time after load-cessation. This is the first report which examines the long-term structural 'memory' of functional adaptation to disclose that loading exerts persistant benefits in bone structure that are retained despite the long-term absence of the original osteogenic stimulus.