Bone Volume is Not Altered with Unloading in the Homozygous Sclerostin Knockout Mouse

+1,2Morse, A; 1McDonald, MM; 1Kramer, I; Kneissel, M; Kelly, NH; 1Melville, KM; 1,4Van der Meulen, MCH; and 1,2Little, DG
+1The Children’s Hospital Westmead, Sydney, Australia, 2University of Sydney, Sydney, Australia, 3Novartis Pharma, Basel, Switzerland, 4Cornell University, Ithaca, USA, 5Hospital for Special Surgery, NY, USA
alysons@chw.edu.au

Introduction
The canonical Wntβ-catenin pathway is a key regulator of bone formation, although its role in mechanotransduction is poorly understood. An endogenous inhibitor of Wnt signaling, SOST, is down-regulated with loading and up-regulated with unloading1. The expression of SOST is mostly specific to osteocytes, which are involved in mechanotransduction. Undertaking an unloading model in homozygous Sost knockout (Sost−/-) mice will aid in understanding the roles that SOST and the Wntβ-catenin pathway have in the response of bone to mechanical load.

Hypothesis
In Sost−/- wild-type mice we expect decreased bone volume in unloaded tibiae compared to control contralateral tibia. We hypothesize inhibition of unloading-induced bone volume loss in the tibiae of Sost+/+ mice.

Methods
Female 10 week old Sost−/- and Sost+/+ wildtype (WT) littermates (N=10 per group) were injected with 0.5U botulinum toxin (BTX, Allergan) into each of the right quadriceps and calf muscles. This resulted in right limb disuse and tibial unloading. Left tibiae served as normal-load controls, as mice were able to maintain otherwise normal activity. Mice were monitored daily for the first three days post BTX injection, and then weekly, to ensure right hind-limb disuse. The study was approved by the Institutional Biosafety Committee and Animal Ethics Committee. End-point was 2 weeks post BTX injection, with bone volume and architecture parameters measured by micro-computed tomography (Skyscan 1174). Trabecular bone was analyzed from a volume of interest (VOI) within the metaphysis, with both primary and secondary spongiosa incorporated and the cortical shell excluded. Cortical bone was analyzed from a mid-diaphyseal VOI. Statistical analysis was performed using paired samples t-test analysis with a 95% confidence interval. 3D reconstructions were created from both the trabecular and cortical VOIs.

Figure 1: Representative 3D µCT images of Sost−/- and WT (Sost+/+) unloaded and control tibial volumes of interest. A. Mid-diaphyseal VOI of cortical bone. B. Metaphyseal VOI of trabecular bone.

Results
Both cortical bone volume and cortical thickness were reduced by 6% following unloading in the WT tibiae compared to WT control tibiae (p<0.01). This bone loss translated to an 8% decrease in polar moment of inertia in unloaded WT tibiae compared to control (p<0.05). In contrast, cortical bone volume, cortical thickness and polar moment of inertia within the mid-diaphysis was not changed between unloaded and control tibiae in Sost−/- mice (Figure 1A).

Trabecular bone volume within the tibial metaphysis was reduced by 47% in unloaded WT tibiae compared to control WT tibiae (p<0.01, Figure 1B). In contrast, trabecular bone volume was not changed between unloaded and control Sost−/- tibiae. Bone volume fraction (BV/TV) was decreased by 49% in unloaded WT tibiae (p<0.01), due to the decrease in bone volume but no change in total volume. BV/TV in unloaded Sost−/- tibiae was also significantly reduced by 8% (p<0.01). However, this was due to a 5% increase in total volume (p=0.01) and not a change in bone volume. Overall, the decrease in BV/TV in the WT unloaded tibiae was six-fold greater than the decrease in Sost−/- unloaded tibiae.

Trabecular architecture was also analyzed within the metaphyseal VOI, with trabecular thickness 11% decreased and trabecular number 43% decreased in unloaded WT tibiae compared to control (p<0.01). In contrast trabecular thickness and trabecular number were unaltered in the unloaded tibiae of the Sost−/- mice.

Discussion
Unloading through disuse of the right tibiae was achieved with BTX. Significant bone loss in both cortical and trabecular bone was demonstrated with unloading in WT mice. This unloading-induced bone loss was inhibited in the Sost−/- tibia indicating a significant role for SOST, and so the Wntβ-catenin pathway, in mechanical induced modulation of bone. Loading studies underway in Sost−/- mice will further elucidate mechanical induced bone modulation mediated by SOST.

An interesting change to the unloaded Sost−/- tibia was an increase in total volume in the metaphysis, leading to a slight decrease in BV/TV. Early investigations suggest this change may reflect primary spongiosa which formed during the unloaded period. It is unknown whether this total volume increase is a result of bone resorption at this site, or rather a reduction in new bone formation at the growth plate. Histomorphological bone formation analysis will assist in answering this question.

Future studies will investigate whether other Wntβ-catenin pathway inhibitors are also involved in mechanical induced modulation of bone. However, the strong inhibition of unloading-induced bone loss with SOST knockout in this study indicates an essential role for SOST in mechanical induced bone modulation.

Significance
The knock-out of SOST was able to inhibit unloading-induced bone loss. This study suggests an essential role for SOST in the mediation of bone loss in response to unloading. SOST modulation may prove applicable to clinical situations of disuse osteopenia.

References

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