FSTL3 mediates exercise driven bone formation.

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Introduction: Timely healing of patients with fractures or bone defects of the hand is of utmost importance, both to reduce the economic burden of lost work-days, as well as to maximize function of the post-injured hand. Thus, adjuvant therapies that expedite healing are much needed. Exercise is known to promote remodeling in bones that are directly biomechanically stimulated, yet little is known about its secondary effects at non-stimulated sites. Furthermore, the role of follistatin-like 3 (FSTL3), a mediator of exercise-driven bone formation, has not been previously shown. The objective of this study was to examine the influence of biomechanical signals generated by exercise and FSTL3 in inducing bone formation in distant, non-stimulated regions.

Methods: Four millimeter diameter, full thickness calvarial defects were made in C57/BL and FSTL3 knockout mice. Subsequently, tissue engineered scaffolds containing bone marrow derived mesenchymal stem cells (BMSCs) were placed in the defects and the incisions closed. Mice (n=5/gr) were either subjected to: (i) no treatment; (ii) gentle treadmill walking; (iii) subcutaneous injection of BMP-2 or (iv) injection of FSTL3. The calvaria were harvested 6 or 12 weeks later and examined for bone formation by μCT, histology, and immunohistochemistry. Statistical analysis was performed by one-way ANOVA and Tukey's post hoc test.

Results: Bone formation to some extent was observed in all defects implanted with BMSC-containing scaffolds. However, TW induced significantly greater (4 ± 0.6 fold) vascularized ossification in the defects. A 2.2 ± 0.7 fold increase in bone formation was observed in mice injected with FSTL3; likewise, an increase in bone formation (2.9 ± 0.6 fold) was observed in mice injected with BMP-2. Histologically, the bone formation in mice subjected to TW demonstrated well-integrated bone. However, bone integration in response to FSTL3 or BMP-2 was incomplete 12 wks following implantations. Importantly, TW failed to induce bone formation in *Fstl3*-/- mice.

Conclusions: Our data suggest that biomechanical stimulation is a potent inducer of bone formation, and its effects are systemic as evidenced by bone formation in defects that are not directly stimulated. Patients could potentially benefit from prescribed exercise programs. Furthermore, the identification of FSTL3 as an inducer of bone formation provides a novel paradigm for future therapies aimed at augmenting bone formation, especially in large or difficult to heal bone defects.