Utilizing Dynamic Histomorphometry to Evaluate Bone Formation in Mouse Femurs Following Myostatin and Activin-A Inhibition

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Introduction

Osteogenesis Imperfecta (OI) is a heritable connective tissue disorder that affects 1:15,000-20,000 births (1). OI, known commonly as brittle bone disease, occurs primarily due to mutations in the type I collagen genes, COL1A1 and COL1A2. OI symptoms include multiple and repeat fractures, malformation of long bones, scoliosis, blue sclera, low muscle mass, and inherent muscle weakness. OI has four major types. Types I/IV are the most common and least severe; type II is most detrimental and results in perinatal death; and type III is the most severe form compatible with life (2). Pervasively, OI type III results in non-ambulation.

Bisphosphonates are currently the most common treatment for OI. They inhibit osteoclasts from absorbing bone tissue, leading to an accumulation of bone tissue. Bisphosphonates are particularly ineffective for young people. Due to the inhibition of osteoclasts, bone remodeling during adolescence is stalled, which is detrimental for long-term bone health.

Bone exists in a biomechanical/biochemical equilibrium with muscle, responding to changes in muscle size and force. Myostatin negatively regulates muscle mass, thus decreasing circulating myostatin increases muscle mass which increases the force exerted on bone, culminating in increased bone mass (3). Activin A regulates osteoclast differentiation, and its inhibition is associated with increases in bone mass (4). In a previous study, a soluble activin receptor type IIB-mFc (sActRIIB-mFc) fusion protein was used to inhibit myostatin statistically improving bone mass, microarchitecture, and strength (4). The exact mechanism of sActRIIB-mFc remains unclear, and has also displayed undesirable results in clinical trials, likely because sActRIIB-mFc binds with multiple unintended targets besides myostatin and activin A (5). ***If inhibiting myostatin or activin A, in isolation, is shown to be effective at increasing bone mass, microarchitecture, and strength, it's possible that the use of sActRIIB-mFc can be avoided, circumventing undesirable side effects***.

This study hypothesizes that inhibiting only myostatin or activin A will increase femoral mineralizing surface (MS), mineral apposition rate (MAR), and bone formation rate (BFR) compared to a control antibody, which are indicative of increased osteoblast activity and bone growth. Narrowing in on femoral cortical MS, MAR, and BFR in Wt mice treated with myostatin or activin A antibodies will help elucidate the mechanistic impact of the antibody treatment. This study intends to lay the foundation for the identification of a treatment with more precision than sActRIIB-mFc.

Figure 1: OI classification

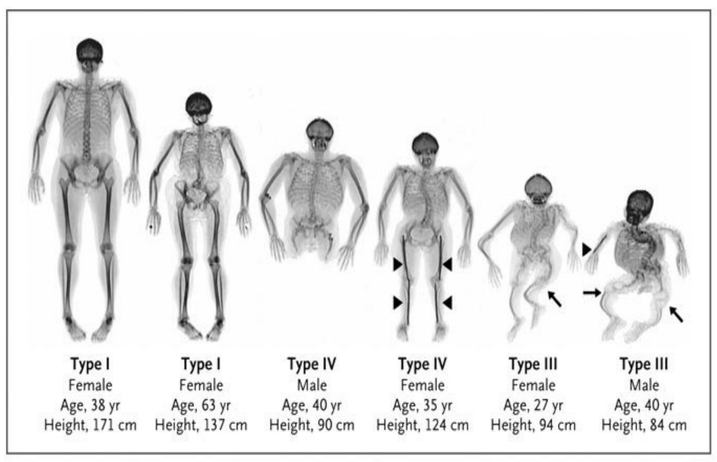

Figure 1: The Sillence classification of osteogenesis imperfecta. The Sillence classification system identifies four main types of OI ranging from mild to severe with type I being mild; type II, perinatally lethal; type III, severely deforming, and type IV moderately deforming (2).

Figure 2: Signaling pathways of Myostatin and Activin A

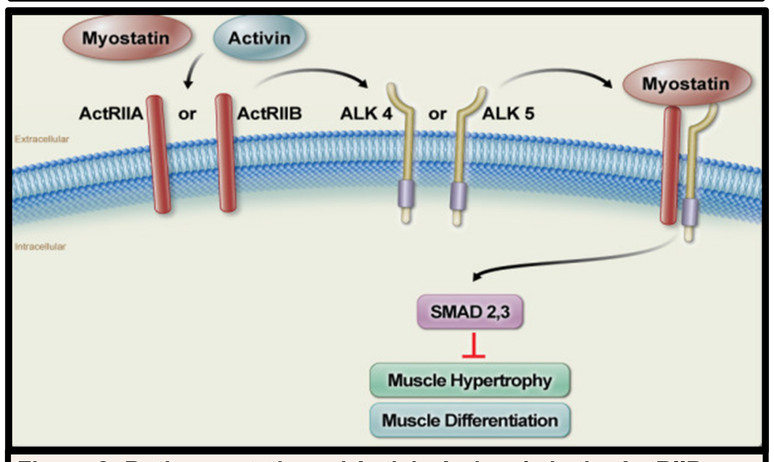

Figure 2: Both myostatin and Activin A signal via the ActRIIB receptor
Lee, S. J., & Glass, D. J. (2011). Treating cancer cachexia to treat cancer. *Skeletal muscle*, *1*(1), 2. doi:10.1186/2044-5040-1-2

Methods

Five-week-old male and female wildtype mice were treated with a monoclonal anti-myostatin antibody, anti-Activin A antibody, or a control antibody (Regeneron Pharmaceuticals) for 11 weeks. All mice were sacrificed at 16.5 weeks of age, when mice display peak bone mass. A calcein label and an alizarin red label were administered 10 and 3 days prior to euthanization, respectively. Left femurs were removed, fixed in 10% neutral-buffered formalin (NBF) for 48 hours, transferred to 70% ethanol and then embedded in methyl methacrylate (MMA). 100-µm sections of bone were cut at the femoral midshaft using a Leica 1600 saw microtome. Using a Leica SP8 spectral confocal microscope, a series of cross-sectional images of the femurs (10× magnification) was obtained. Fluorescent labeling distance and the distance between the two fluorescent labels was quantified using ImageJ software. Cross section IDs were then grouped in a double-blind fashion and the significance of MS, MAR, and BFR between the two treatments and the control was determined using Anova.

Figure 3: Femur Cross Sections Stained with Alizarin Red and Calcein

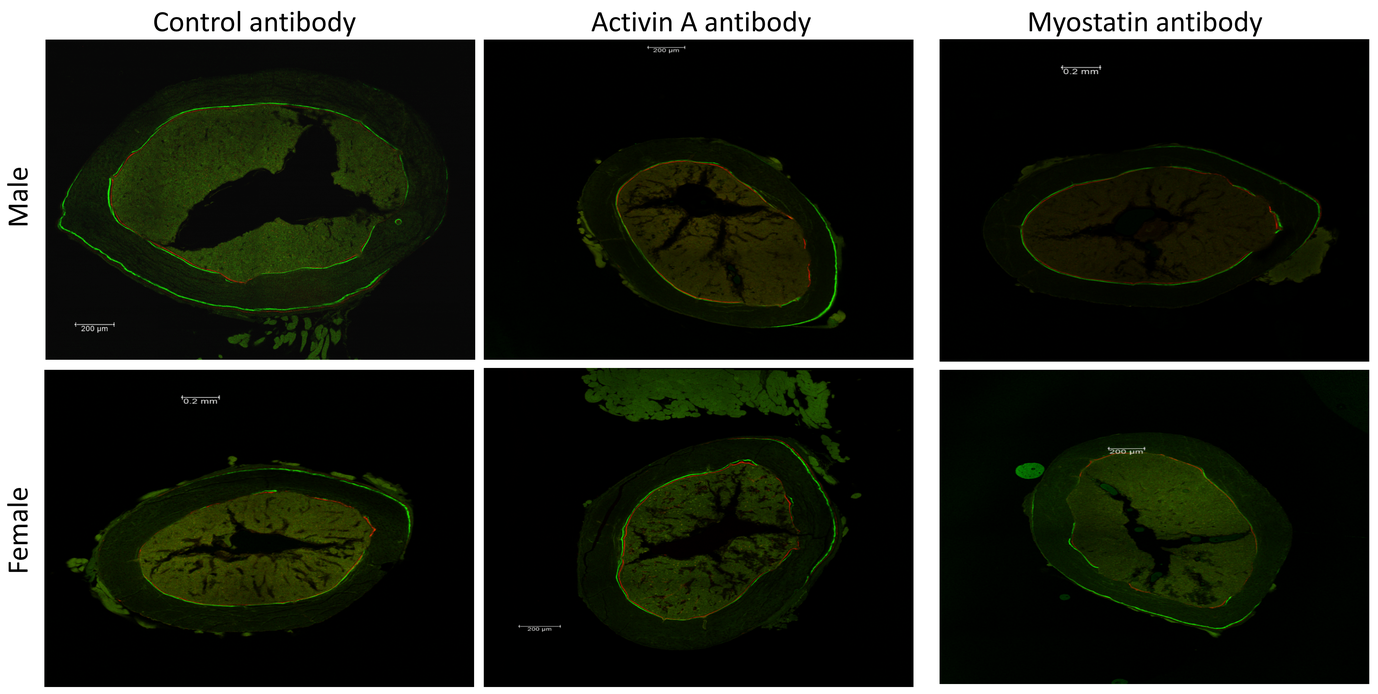

Figure 3:

Fluorescently labeled cortical cross sections in wildtype mouse femurs, treated with a control, anti-activin A antibody, and anti-myostatin antibody. All three were tested in both male and female mice.

Figure 4:

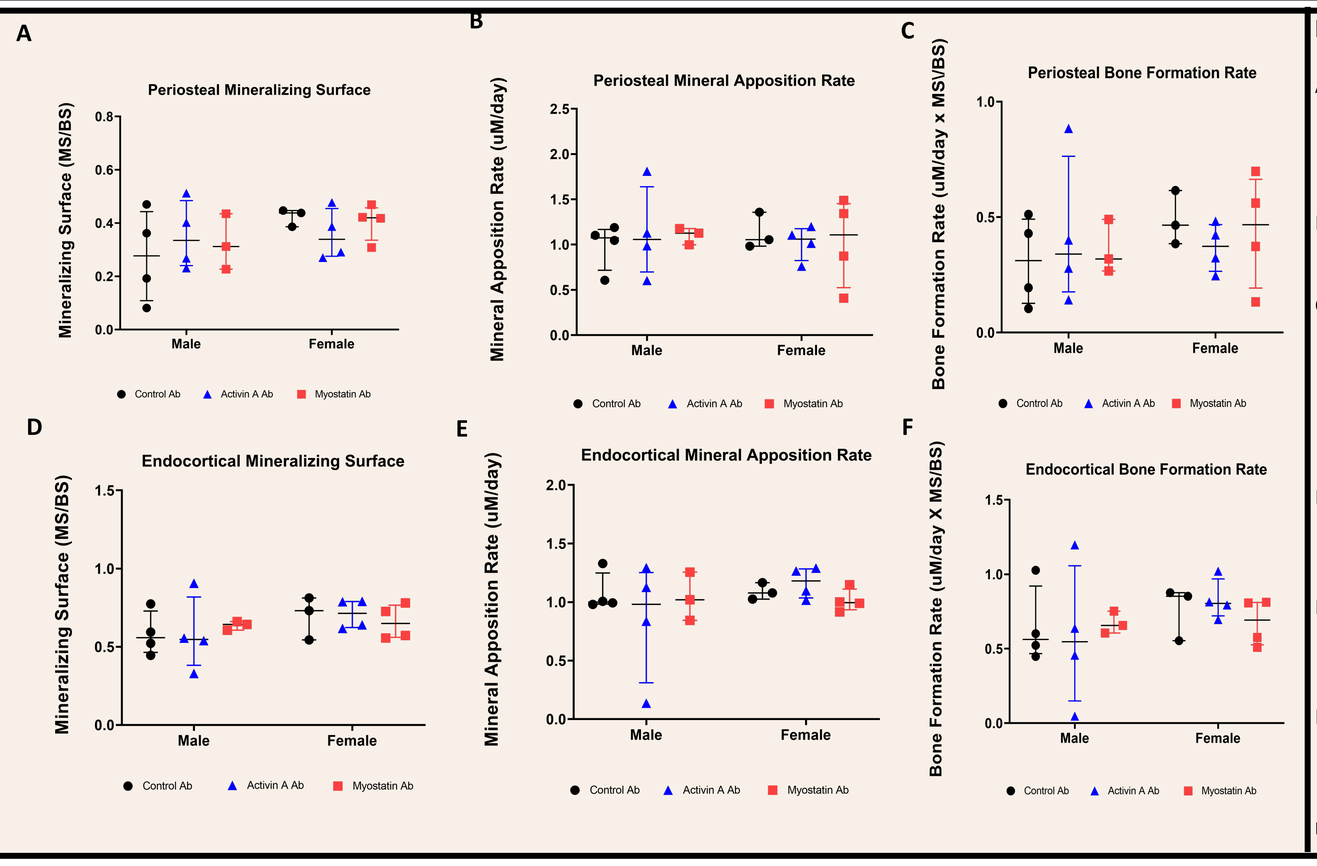

Figure 4:

A. Both male and female Wt mice have equivalent periosteal MS regardless of control, anti-myostatin or anti-activin A antibody treatment.

B. Male and female display no significant difference in MAR.

C. No significant difference displayed in male or female across all three treatments. Male Activin-A Ab displays a high degree of variance, but the average is not significant from the Control Ab.

D. No significant difference displayed in MS for male or female mice across the three treatments.

E. Differences in MAR among the three treatments for both male and female are nearly identical.

F. Endocortical BFR between male and female for each treatment are not significantly significant.

n= 3-4 for all groups

Conclusions

- Neither of the two treatments displayed significant differences in MS, MAR, or BFR compared to the control in male or female mice.
- This suggests that in isolation, myostatin and activin-A, do not significantly change bone growth or, potentially, osteoblast activity.
- However, preliminary data of other studies conducted in the lab suggests that a combination of activin-A and myostatin are necessary to display the improved bone mass, microarchitecture, and strength associated with sActRIIB-mFc treatment, suggesting that a myostatin antibody or an activin-A antibody, in isolation, are not potent enough to display increased bone growth or osteoblast stimulation. Continued research on both components will be required.

Future work

- Continue study with combination of activin A and Myostatin inhibition as previous studies have shown combo treatment is most statistically significant
- Utilize static histology to evaluate osteoblast and osteoclast activity in mice treated with myostatin antibody or activin-A antibody
- Conduct a similar study in an OI mouse model

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Acknowledgements

- Agriculture Institute- CAFNR Undergraduate Research Internship
- University of Missouri School of Medicine Child Health Research Institute
- Kansas City Consortium on Musculoskeletal Diseases (KCMD) Collaborative Research for Neuromuscular /Musculoskeletal Disorders pilot grant
- Washington University Institute of Clinical and Translational Sciences (ICTS)
- NIH Clinical Translational Sciences Award (CTSA Grant Number UL1TR002345)
- Regeneron Pharmaceuticals Inc