The Effects of Muscular Stretch Training on Vasculature in the Proximal Tibial Metaphysis

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Background

Four weeks of daily stretching of the calf muscles has been shown to increase total volume of the proximal metaphysis of the tibia in old rats; however, bone volume did not increase, and trabecular connectivity decreased slightly, suggesting that the volume of other tissue is increasing within the bone. Daily muscular stretching also increased bone blood flow to the proximal metaphysis, possibly stimulating angiogenesis (the formation of blood vessels) in that region of the bone. Increased flow and angiogenesis could also increase interstitial fluid flow within the proximal metaphysis, stimulating bone remodeling. The purpose of this study was to determine if daily muscular stretching causes an increase in vascular tissue proximal metaphysis of rat tibias.

Hypothesis

Bone vascular volume will increase in the proximal metaphysis of the tibia bone from legs in which the calf muscles were stretched daily.

Methods

Prepping, Cutting, and Staining

The left calf muscles of old rats were stretched daily for 4 weeks, 5 days a week, and 30 min/day using a custom splint. Left (stretched, n=5) and right (non-stretched, n=5) tibias from each rat were decalcified using 14% EDTA for 2 weeks. Tibias were frozen in OCT and sectioned with a cryostat. Cutting of 13 µm longitudinal sections was initiated ~650 µm from the medial surface of the tibial metaphysis. 20 sections were cut from each tibia and stained with lectin to identify endothelial/vascular tissue. 10 sections were cut from each tibia and used as controls (no lectin staining). DAPI stain was used to identify nuclei.

Imaging and Analysis

Sections were imaged using a fluorescent microscope at 4X magnification. The volume of lectin-stained tissue was computed with a custom Python program that randomly analyzed the fluorescent structures to yield an average vascular volume from each image set. The volume of non-stretched and stretched tibias were compared using a paired T-test.

Results

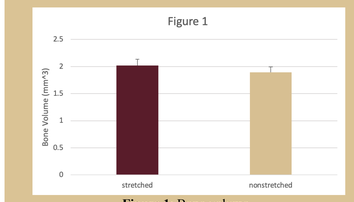

Figure 1: Bone volume

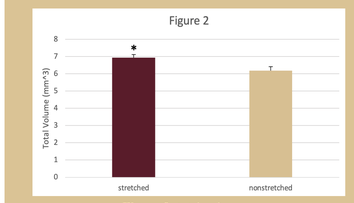

Figure 2: total volume

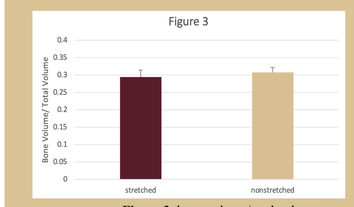

Figure 3: bone volume/total volume

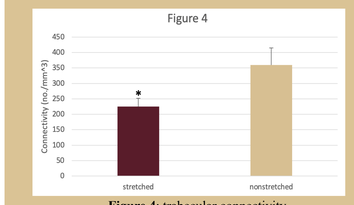

Figure 4: trabecular connectivity

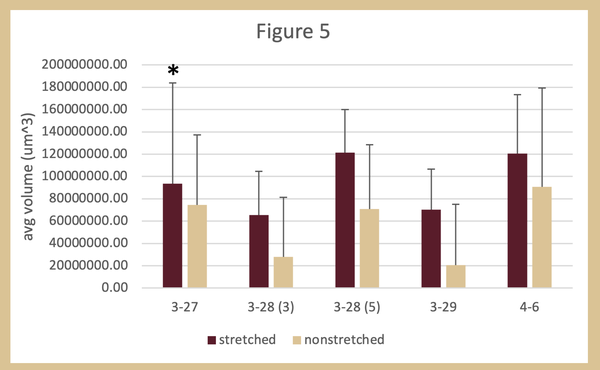

Figure 5: represents the average vasculature volume of each section taken from stretched vs non stretched rat legs. All are $p<0.01$ except for rat 3-27

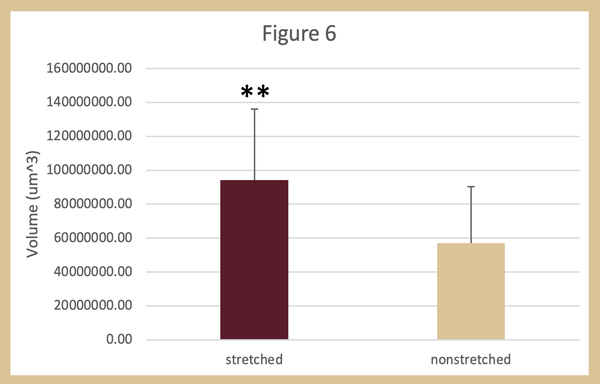

Figure 6: represents the total average vasculature volume of a section from all stretched vs non stretched rat legs. On average, there was an increase in vasculature volume by 65%.

* $p<0.05$ stretched vs non-stretched limb, ** $p<0.01$ stretched vs non-stretched limb

Images

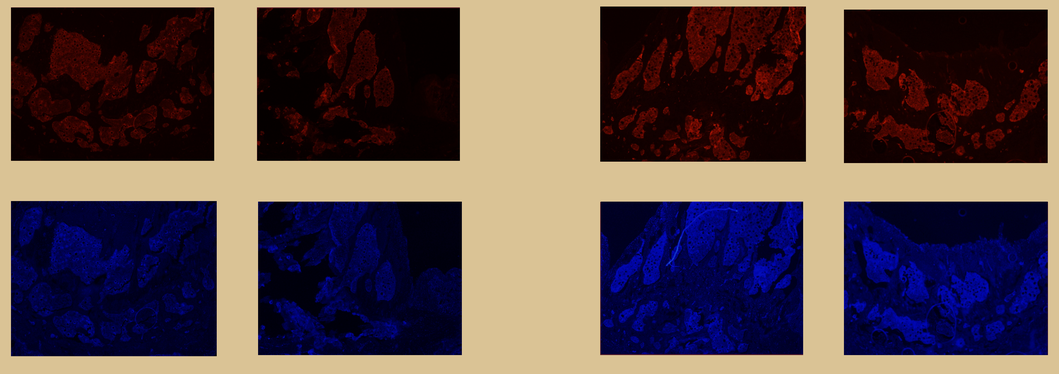

Image 1: lectin from stretched leg 1, **Image 2**: DAPI from stretched leg 1, **Image 3**: lectin from non-stretched leg 1**, Image 4**: DAPI from non-stretched leg 1, **Image 5**: lectin from stretched leg 2, **Image 6**: DAPI from stretched leg 2, **Image 7**: lectin from non-stretched leg 2**, Image 8**: DAPI from non-stretched leg 2.

Findings

- Total bone volume of the proximal metaphysis increased in response to four weeks of daily muscle stretching.
- Bone volume/total volume of the proximal metaphysis of the tibia did not change in response to four weeks of daily muscle stretching.
- Trabecular connectivity volume of the proximal metaphysis of the tibia decreased in response to four weeks of daily muscle stretching.
- Vascular volume of the of the proximal metaphysis of the tibia increased in response to four weeks of daily muscle stretching.

Conclusion

Our findings suggest that four weeks of daily muscular stretching stimulates bone remodeling in the proximal metaphysis of the tibia; however, four weeks of stretching results in an increase in vascular volume without an increase in bone volume. Further study is needed to determine if a longer period of muscular stretching increases trabecular bone volume within the tibial metaphysis.

Acknowledgments

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