Monitoring Exercise- and Diet-Induced Changes in Tibial Blood Perfusion with Laser Doppler Flowmetry in Mice

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Vascular function plays a critical role in bone development and repair, and exercise, which stimulates vascular growth and performance [1], may ameliorate detrimental bone changes associated with diabetes or stroke [2]. Measuring changes in bone vasculature *in vivo* is challenging, and current techniques to monitor functional changes in bone perfusion longitudinally are ineffective. Laser Doppler flowmetry (LDF) can quantify vascular changes in murine tibiae and provide a functional measure of bone perfusion beyond vascular density from histology or growth factor concentrations in serum [3]. However, a previous study reported local inflammation lasting several months, which could confound longitudinal flow measurements [3] and alter normal gait. We performed a less invasive LDF procedure to minimize inflammation and allow longitudinal perfusion measurements. We hypothesized our procedure would detect changes in tibial blood perfusion of young adult male mice with exercise and high fat diet-induced obesity without altering gait.

Endpoint LDF measurements detected increased tibial perfusion with both treadmill exercise (exercise vs. sedentary) and diet (obese vs. lean) following a nine-week intervention. Relative to sedentary, exercise increased perfusion in both lean (+38%) and obese (+21%) mice. In sedentary mice diet-induced obesity increased perfusion 24%. Exercise-induced changes in endpoint serum VEGF concentrations were detectable only in obese (not lean) mice.

We performed repeated LDF measurements 10 days apart in sedentary, lean mice (no intervention) and found no significant changes in perfusion over time, as expected. Further, no visual signs of inflammation were observed, as assessed by incision site appearance and size. Hindlimb mechanics were examined with high-speed video monitoring of treadmill exercise for a week following the LDF procedure to determine if gait was altered by LDF. No changes in gait mechanics (phase dispersion, duty cycle) were found in the affected limb, and mice were able to complete their exercise regimens with no visible signs of discomfort.

Initial results indicate that the modified LDF procedure detects changes in blood perfusion within bone beyond what can be measured from serum VEGF alone and is appropriate for longitudinal measurements. Future work will correlate LDF perfusion with osteovascular architecture and cellular/molecular signaling. LDF will be used to assess therapeutic potential of particular exercise regimens in preventing fracture with animal models of lifestyle diseases that cause vascular dysfunction, such as obesity and stroke.

References:

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