Skeletal perfusion measurements during graded exercise in rat hind limb

K. I. Marro[1], O. M. Hyyti[1]
[1]Radiology, University of Washington, Seattle, WA, United States

Abstract
The goal of this study was to develop a technique to measure skeletal muscle perfusion dynamics during graded exercise. Single voxel perfusion measurements were acquired at different metabolic loads by varying the force of stimulated contractions in rat hind limb. The results show a significant difference in the amplitude of the perfusion response. In addition the time course of perfusion changes during stimulation and recovery can be easily determined.

Introduction
The dynamics of capillary-level perfusion are critical to the function of skeletal muscle under normal conditions and can be compromised in a variety of disease states such as diabetes, congestive heart failure and peripheral vascular disease. Yet little is known about the complicated relationship between perfusion and metabolic demand due to the difficulty of obtaining reliable perfusion measurements in vivo. Accurate non-invasive measurements of perfusion during graded exercise could have wide application in both basic science and clinical settings.

Methods
Experiments were conducted on a single hind limb in each of 8 male rats (275-325 g) anesthetized with isofluorane. Contractile force was measured via a force transducer connected to the foot. A bipolar electrode was surgically placed around the ligated sciatic nerve to induce contractions of all lower hind limb muscles. Excitation pulses of 0.1 msec duration were applied at variable frequencies in 250 ms long pulse trains at a rate of 1 train every 5 seconds. Graded exercise was induced by actively adjusting the frequency of the excitation pulses as necessary to maintain contractile forces of 20, 40 and 60% of the maximum stimulated contraction (MSC).

The animals were placed in a 4.7 T Bruker magnet with a Varian Inova console running VNMR 6.1c. Perfusion measurements were acquired using the FAWSETS pulse sequence (1) at rest, during ischemia (induced by an inflatable cuff placed around the upper limb) and during the stimulation and recovery periods. Acquisitions during the stimulation period were interleaved between the contractions. The perfusion excitation protocol consisted of 4 seconds of continuous RF with a magnitude of about 100 mgauss. This was followed by a slice-selective 180-degree pulse to localize the FID in a 5 mm thick (~0.4 ml) volume at the midpoint of the lower limb. Symmetric crusher gradients sandwiching the 180-degree pulse eliminated the signal contribution from faster flowing arterial water in larger vessels. This excitation protocol results in a signal proportional to capillary perfusion plus a small contribution from residual tissue water. To eliminate the residual tissue signal, the mean of the 10 FID's acquired during the ischemic period were subtracted from all the FID's in the rest-stimulation-recovery cycle. The amplitudes of the perfusion response at 20, 40 and 60% MSC were determined by averaging the last 2 minutes of acquisitions during the stimulation periods.

Results
The pattern of the perfusion response was similar at all three contractile forces (Fig 1): a small but steady signal at rest (A), a distinct drop to essentially zero during ischemia (B), a return to slightly higher than resting signal immediately following ischemia (C), a gradual (~2 min) rise to steady state during the stimulation period (D) and a slower (~5 min) return to resting levels during the recovery period (E). The amplitude of the perfusion response, given in Fig 2 as mean (SD), increased with increasing contractile force.

Figure 1

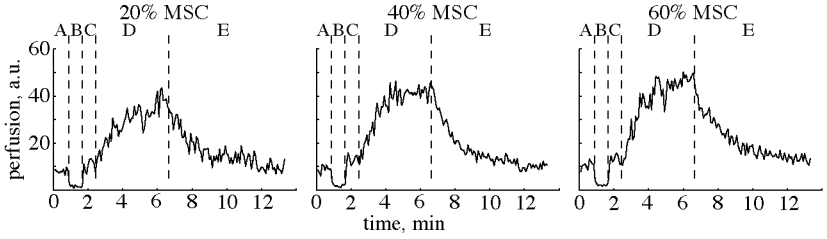
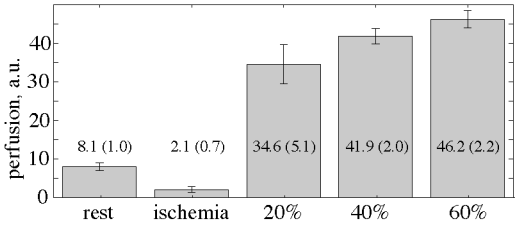


Figure 2



Conclusion
The FAWSETS pulse sequence in combination with an appropriate exercise protocol can be used to monitor changes in perfusion during graded exercise. The sequence is sensitive enough to measure resting perfusion in a 0.4 ml volume with a single acquisition (less than 5 sec) and to measure the perfusion response to different metabolic challenges. Temporal resolution is sufficient to monitor the time course of perfusion changes during stimulation and recovery. This technique, in combination with phosphorous NMR, could be used further the understanding of the link between perfusion and cellular energetics in normal and diseased skeletal muscle.

References:
1. K. Marro, J Magn Reson 1997;124(1):240-244.