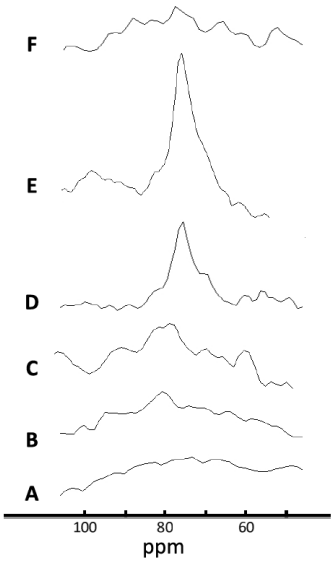
Myoglobin Contribution to the Near Infrared Signal in Exercising Skeletal Muscle

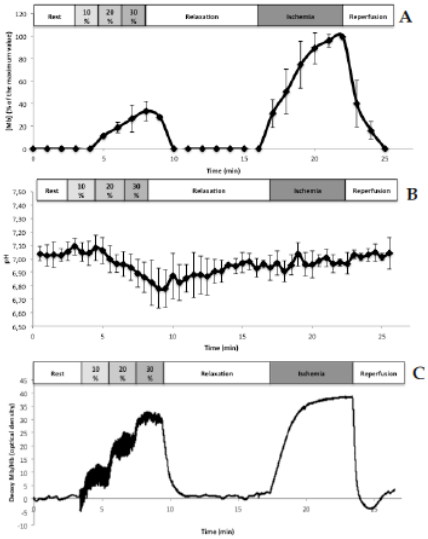
David Bendahan[1], Benjamin Chatel[1], and Thomas Jue[2]
[1]CNRS, CRMBM, Aix-Marseille University, Marseille, France, [2]Biochem & Mol Medicine, University of California, Davis, CA, United States

BACKGROUND: Many researchers and clinicians use routinely near Infrared spectroscopy (NIRS) to measure tissue oxygenation and have presumed hemoglobin (Hb) as the signal source. In muscle, however, both Mb and Hb give rise to overlapping NIRS signals and the relative contribution of each species remains moot. The orthodox view envisions Hb as the main signal source, whereas comparative analysis of the ^{1}H NMR intracellular myoglobin (Mb) and NIRS signals from human calf muscle has suggested that NIRS tracks mainly Mb saturation (1). Without a clear understanding of the NIRS signal contribution in exercising muscle, no scientific-based consensus can emerge to resolve many conflicting interpretations.

The present combined NMR-NIRS study has interrogated the relative contribution of Mb and Hb to the NIRS signal in exercising human finger flexor muscles. Indeed, Mb does contribute predominantly to the NIRS signal, consistent with physiological estimates based on the microvasculature volume and Hb concentration vs. cellular volume and Mb concentration. With increased work, respiration increases but cellular O_2 levels decrease progressively, as reflected in the Mb signal (2). Because NIRS can define the rapid changes in the cellular oxygenation, the combined NIRS and interleaved ^{1}H/^{31}P signal acquisition scheme provides insight into the dynamic metabolic changes in PCr, ATP, Pi, pH, and deoxy Mb during muscle contraction. The rapid O_2 decline at the initiation of muscle contraction accompanies a PCr consumption, a Pi accumulation, and an intracellular acidosis. ATP remains constant. Even at a relatively high level of oxygenation, the rapidly decreasing pH indicates that the cell has already mobilized glycogen to produce lactate. No anaerobic threshold appears to trigger lactate production. Observing the tight metabolic interplay at a defined O_2 level presents a unique opportunity to better understand the complex mechanism controlling the cell response to a surge in energy demand during muscle contraction.

MATERIAL AND METHODS: Five healthy males (age 37 ± 13 yr) volunteered for the study, which was approved by the local ethics committee. For each investigation, the subject inserted his dominant forearm in a Bruker Biospec 47/20 magnet, which rested above a 50 mm ^{1}H/^{31}P double-tuned surface coil. An NIRS optical sensor was placed at the center of the coil for simultaneous NMR/NIRS signal detection. The maximum isometric voluntary contraction for the finger flexor muscles was determined by 3 different trials. The subjects then performed 2-min continuous exercise at 0.33 Hz x 3 different intensities (10, 20 and 30% MVC) for a total 6 min. Post-exercise recovery period lasted for 8-min. A 6-min arterial occlusion (180 mm Hg using a cuff placed around the upper arm) then began, followed by a 3-min reperfusion.





Interleaved ^{31}P and ^{1}H MRS signal acquisitions monitored the high-energy phosphate metabolite, pH and oxygen levels. Both ^{31}P and ^{1}H NMR used a pulse-acquire sequence (250-μs single square pulse, 1.5s repetition time for ^{31}P and a selective excitation of the deoxymyoglobin resonance, at 78 ppm. Data were then processed using the AMARES-MRUI Fortran code using appropriate prior knowledge for the ATP multiplets and myoglobin signal. The final time-resolution was 60s and 30s for the ^{1}H and ^{31}P datasets, respectively. The percentage of Mb desaturation was referenced to the signal intensity at the end of the ischemic period. ^{31}P signals were normalized to the fully relaxed signals. A continuous-wave near-infrared spectrometer (Oxymon, Artinis) recorded the NIRS signals using optical fibers positioned 4.5 cm apart on the forearm muscle.

RESULTS: Fig 1 shows ^{1}H NMR spectra from the muscle during a) rest b) 10% MVC c) 20% MVC d) 30% MVC e) ischemia f) reperfusion. The signal at 78 ppm arises from proximal histidyl $N_{\delta}H$ signal of deoxy Mb, while the small upfield peak at 73 ppm originates from the β proximal histidyl $N_{d}H$ signal of deoxy Hb. The deoxy Mb signal increases during exercise and ischemia but is not measureable during relaxation and reperfusion (2). The deoxy Mb signal dominates over the deoxy Hb signal. Fig 2 shows the course of the deoxy Mb, pH, and the NIRS signal assigned to the deoxy Mb/Hb during the exercise protocol: rest, 10% MVC, 20% MVC, 30% MVC, relaxation, ischemia, and reperfusion (A) the NMR changes in the deoxyMb signal (B) pH derived from the ^{31}P signal of Pi (C) the NIRS signal of deoxy Mb/Hb signal. Even at moderate exercise, when Mb shows only 20% desaturation, pH has started to decline, consistent with the presence of lactate production (3).

CONCLUSION: The results indicate that 1) as work and respiration increase with exercise intensity, the intracellular O_2 level drops progressively 2) the NIRS signal of muscle reflects predominantly the Mb saturation and not Hb saturation 3) lactate production has started even under well oxygenated cellular conditions 4) a combination of NIRS and NMR approach can provide unique insights into the control of bioenergetics in healthy and compromised muscle.

REFERENCES: 1. Tran TK et al. AJPhys 276, 1682,1999. 2. Mole PA et al. AJPhys 276, R173, 1999. 3. Hsu A and Dawson, M.J. MResMed. 44, 418, 2000.