Characterization of Metabolic Response to Ischemia in Skeletal Muscle of Non-obese Early Stage Type 2 Diabetic Rats by in vivo 31P MRS and BOLD MRI

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Target Audience
Researchers interested in skeletal muscle metabolism, type 2 diabetes, 31P Magnetic Resonance Spectroscopy (MRS), and Blood Oxygen Level-dependent (BOLD) MRI.

Introduction/Purpose
Type 2 diabetes (T2D) is associated with mitochondrial dysfunction. However, the time course of the development of mitochondrial dysfunction in skeletal muscle is not well defined. While altered mitochondrial energy metabolism during exercise has been observed in diabetic rats [1], another study reported normal post-exercise recovery of phosphocreatine (PCr) in early stage T2D patients [2]. In current study, we aimed to investigate metabolic response to ischemia in skeletal muscle of non-obese early stage T2D rats.

Methods
7 week old, non-obese T2D Goto-Kakizaki (GK) rats (male, n=5) and their age-matched controls (n=5) were scanned in a 9.4T Bruker horizontal scanner (Billerica, MA). Ischemia was induced by inflation of a home-made cuff that was placed at the thigh. The 300 mmHg cuff inflation lasted for 25 min. Body temperature was maintained at 34.5±0.5°C during experiment. A home-made 31P saddle coil was used to acquire 31P MR spectra from the tibialis region of the lower leg at baseline, during ischemia, and reperfusion. Acquisition parameters were: TR, 2s; NAV, 64; spectral width, 6000 Hz. 31P MR spectra were processed using MestReNova software with 30 Hz line broadening, polynomial-fit baseline correction, and Lorentzian-Gaussian curve fitting. Areas under each resonance peaks were calculated and normalized to its baseline value. pH was calculated from the relative chemical shift of Pi [3]. BOLD images were also acquired from an axial slice of the lower leg using a FLASH sequence with the following parameters: TR, 500 ms; TE, 7 ms; flip angle, 40°; NAV, 1. BOLD signal was calculated as the mean pixel intensity within an ROI that encompassed the whole calf muscle. 31P spectra and BOLD images were acquired in an interleaved fashion. Acquisition time for each spectrum/image set was 3 min 12 s.

Results
The body weights of GK rats were significantly lower than the controls at the time of scan (179.2±24.3 g vs. 219.5±44.5 g, p<0.05). At the end of 25-min ischemia, PCr reduction in diabetic rats was less pronounced than that in the controls (-86.1±1.2% vs -92.34±1.67% relative to baseline, Fig. 1, *p<0.05). Diabetic rats also showed a smaller increase in Pi than the controls (628.44±98.94% vs 1007.13±233.47%, Fig. 1, *p<0.05). During reperfusion, Pi and PCr changes were the same between diabetic rats and the controls. There is a trend of higher pH for the diabetic group throughout baseline (7.32±0.17 vs 7.08±0.09, p<0.05), ischemia (6.86±0.11 vs 6.74±0.07, p<0.05), ischemia recovery, and end perfusion periods. Maximal BOLD signal decrease during ischemia was significantly higher for diabetes group (-13.86±4.56%) compared to control group (-5.50±1.91%, p<0.05). Under ischemia, signal reduction in BOLD image was found to be inversely correlated with increase in Pi for control group. In contrast, the BOLD image appears independent from Pi for GK rats (Fig. 2).

Discussion & Conclusion
Consistent with an earlier study on T2D patients [2], PCr recovery after ischemia was the same for diabetic and control rats, suggesting that early stage T2D is not necessarily associated with mitochondrial dysfunction. The more pronounced BOLD signal reduction during ischemia is likely associated with higher oxygen extraction in diabetic rats, leading to slower PCr depletion and less Pi increase.

References

Figure 1. Changes in Pi and PCr during ischemia and reperfusion.

Figure 2. Higher Pi signal corresponded to lower oxygen utilization (lower absolute change) for control group, whereas the diabetes group, with more oxygen utilization, had smaller Pi signal change.