Progression of Skeletal Muscle Dysfunction Assessed by 31P MRS and BOLD MRI in Non-obese Type 2 Diabetic Rats

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Target Audience

Researchers interested in skeletal muscle metabolism, type 2 diabetes mellitus (T2DM), ^{31}P Magnetic Resonance Spectroscopy (MRS), and Blood Oxygen Level-dependent (BOLD) MRI.

Introduction/Purpose

Mitochondrial dysfunction in skeletal muscle was observed in rat models of type 2 diabetes mellitus (T2DM)[1] and diabetic patients. However, other human studies reported unaltered mitochondria function. These conflicting observations could be related to a later stage manifestation of metabolic dysfunction in skeletal muscle of T2DM. Furthermore, the progression of mitochondrial dysfunction in non-obese T2DM has not been reported. In this study, interleaved ^{31}P MRS and BOLD MRI were performed to evaluate the response of skeletal muscle to ischemia/reperfusion at 12 and 18 weeks of age in a rat model of non-obese T2DM.

Methods

Non-obese T2DM Goto-Kakizaki (GK) male rats were scanned at 12 weeks (n=6) and 18 weeks (n=7). Wistar control rats were scanned at 22 weeks (n=4) on a 9.4T Bruker horizontal scanner (Billerica, MA). Body temperature was maintained at 35.5±0.5°C during the experiment. A home-made cuff was used to induce occlusion of the femoral artery at the thigh. Interleaved ^{31}P MRS and BOLD MRI scans were performed at baseline, during 26 min ischemia and 26 min reperfusion. A Bruker ^{1}H rat coil was used to acquire BOLD images of an axial slice of the lower leg using the following acquisition parameters: TR, 500 ms; TE, 7 ms; flip angle, 40°; FOV, 4cm×4cm; matrix size, 128×128; NAV, 1 average. Total acquisition was 64 s for each scan. ^{31}P spectra were acquired using a home-made ^{31}P saddle coil placed around the lower leg. Acquisition parameters were: TR, 2s; flip angle, 90°; spectral width, 6 kHz. At baseline, 128 repetitions of ^{31}P spectra were acquired in 4 min 18 s, followed by the acquisition of 3 BOLD images. During ischemia and reperfusion, 160 repetitions of ^{31}P spectra were acquired in 5 min 22 s, followed by one BOLD scan. This interleaved ^{31}P and BOLD acquisitions were repeated four times during ischemia and four times during reperfusion.

^{31}P MR spectra were processed using MATLAB-based software. 4 repetitions were averaged to achieve a temporal resolution of 8 s. A 30 Hz line-broadening was applied to each averaged FID before Fourier transform. The transformed spectra were manually phase corrected. The area under phosphocreatine (PCr) was calculated and normalized to baseline. The time constant of PCr recovery during reperfusion was estimated by fitting a mono-exponential function to the PCr recovery curve[2].

BOLD images were also processed in MATLAB-based software. Region of interest (ROI) was drawn on BOLD images to include all calf muscles, as well as the tibialis anterior and the gastrocnemius muscles. Signal intensity was calculated as the mean value of all pixels within the ROIs and was normalized to baseline.

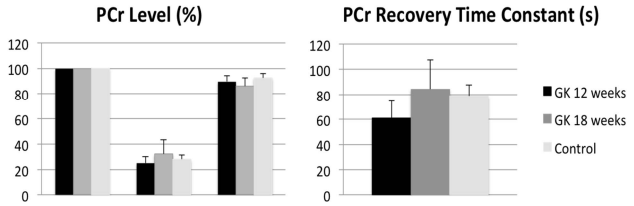

Figure 1. PCr level at baseline, end ischemia and end reperfusion were not significantly different. PCr recovery time constant is significantly longer for 18-week GK rats than that of 12-week GK rats ($p<0.05$).

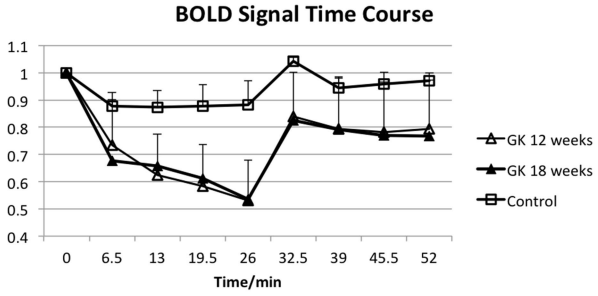

Figure 2. BOLD signal change in the whole muscle during ischemia and reperfusion are identical at 12 weeks and 18 weeks for GK rats.

Results

The body weight was 293.4±37.4, 356.5±19.9, and 518.8±27 g for 12- and 18-week GK rats and 22-week Wistar rats, respectively ($p<0.01$). Baseline-normalized PCr levels at end-ischemia and end-reperfusion were similar among all three groups (Fig. 1). However, the time constant of PCr recovery for 18-week GK rats was significantly longer than that of the 12-week GK rats (60.8±13.9 s vs. 80.4±23.3 s, $p<0.05$, Fig. 1).

Changes in BOLD signal in the whole muscle during ischemia and reperfusion are shown in Fig. 2. The signal change followed the identical trend for the two GK groups. The signal decreased to the lowest level (53.1±14.7% and 53.5±15.7%, respectively) at the end of ischemia, and did not fully recover at the end of reperfusion (79.4±18% and 76.7±11%, respectively). The control group showed a significantly higher oxygen level compared with the GK groups ($p<0.05$). BOLD signal changes in the gastrocnemius muscle were also identical for 12- and 18-week GK rats. However, the tibialis anterior muscle showed a lower oxygenation level for 18-week GK rats as compared to that of 12-week GK rats.

Discussion & Conclusion

The increase of the delayed recovery of PCr after skeletal muscle ischemia, in GK rats from 12 to 18 weeks suggests the development of mitochondrial dysfunction associated with T2DM. The partial recovery of the blood oxygen level indicates that the vascular response to reperfusion maybe impaired at 12 weeks. Furthermore, oxygen delivery and utilization are both compromised in insulin resistant skeletal muscle. Further investigation is needed to compare these changes at later stage of the disease progression with both age- and weight-matched controls.

References

1. R.A. John Challiss et al., AJPEM, 1989. 256, E129-E137.
2. G. Layec et al., NMR in biomedicine, 2013. 26, 1403-11.