Oxygen delivery does not limit mitochondrial function in skeletal muscle of healthy and diabetic rats *in vivo*

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Introduction

Earlier studies have demonstrated an association between the pathogenesis of type 2 diabetes and mitochondrial dysfunction in the skeletal muscle [1, 2]. ^{31}P magnetic resonance spectroscopy (MRS) provides a non-invasive tool to monitor the rate of phosphocreatine (PCr) recovery in muscle after exercise, which is a measure of *in vivo* muscle oxidative capacity. *In vivo* muscle oxidative capacity is determined by mitochondrial content and intrinsic mitochondrial function (together termed intrinsic mitochondrial capacity), but in addition also depends on the supply of oxygen (O_2). In order to infer information of intrinsic mitochondrial capacity from measurements of PCr recovery, it is therefore essential to ensure that O_2 supply is not a limiting factor. Haseler et al. [3, 4] demonstrated that in sedentary subjects PCr recovery is determined by the intrinsic mitochondrial capacity, but that in exercise-trained subjects with a high intrinsic mitochondrial capacity, PCr recovery is limited by O_2 supply and is therefore not a good measure for intrinsic muscle mitochondrial capacity. In a recent study it was shown that the blood oxygenation-level dependent BOLD response in muscle of patients with type 2 diabetes was significantly lower than in controls, indicating that O_2 supply might be a limiting factor in PCr recovery in diabetic muscle [5].

Aim: To determine if PCr recovery in diabetic rat muscle is limited by oxygen delivery. To this aim, we measured PCr recovery using ^{31}P MRS under normoxic and hyperoxic conditions in lean, healthy fa/+ and obese, diabetic fa/fa Zucker diabetic fatty (ZDF) rats. To monitor changes in muscle oxygenation during and after stimulation, BOLD imaging was performed in the skeletal muscle.

Materials and Methods

Animals: For this study, lean, healthy fa/+ (n = 6) and obese, diabetic fa/fa (n = 6) ZDF rats (14 weeks of age) were used. Experiments were performed under normoxic and hyperoxic (fractions of inspired oxygen, F_{O2}=0.21 and F_{O2}=1.00, respectively) conditions on separate days (2-3 days apart).

Muscle stimulation: Muscle contractions were induced by electrical stimulation of the *tibialis anterior* (TA) muscle, via subcutaneously implanted electrodes. The force produced by the TA during contraction was measured using a custom built force transducer. The intensity of the stimulation protocol was set to the maximal contractile force measured by the force transducer at the start of the protocol. After a 3-min rest measurement, the TA muscle was electrically stimulated for 2 min followed by a 10-min recovery period. A pulse oximeter (Nonin, Minnesota) was used to monitor O_2 saturation in the blood.

BOLD imaging: BOLD imaging was performed on a 6.3 T horizontal Bruker MR scanner with a circular ^{1}H surface coil (∅ 40 mm) and using a gradient echo EPI sequence (TR/TE= 1000/30 ms, FOV = 4.5 cm) to assess local oxygenation changes in the TA muscle. The region of interest (ROI) for quantification of the signal intensity (SI) was drawn in a high-resolution FLASH image and then superimposed on the BOLD images (Figure 1A). The SI was normalized relative to the average baseline SI determined at rest. The BOLD response was then determined as the SI increase immediately after the stimulation protocol (Figure 1B).

^{31}P MRS: After the BOLD measurement, the same stimulation protocol was repeated and this time ^{31}P MRS was performed on the TA muscle using an ellipsoid ^{31}P surface coil (10/18 mm). ^{31}P MR spectra were acquired using an adiabatic BIR4 pulse with a 90° flip angle, TR = 5 s and 4 averages. ^{31}P MR spectra were fitted in the time domain using AMARES in the jMRUI software package. The recovery of PCr was fitted to a mono-exponential function yielding the PCr recovery rate constant, k_{PCr}. Data are presented as means ± SD. Data were analyzed statistically by applying two-way ANOVA using SPSS. Level of significance was set at $P < 0.05$.

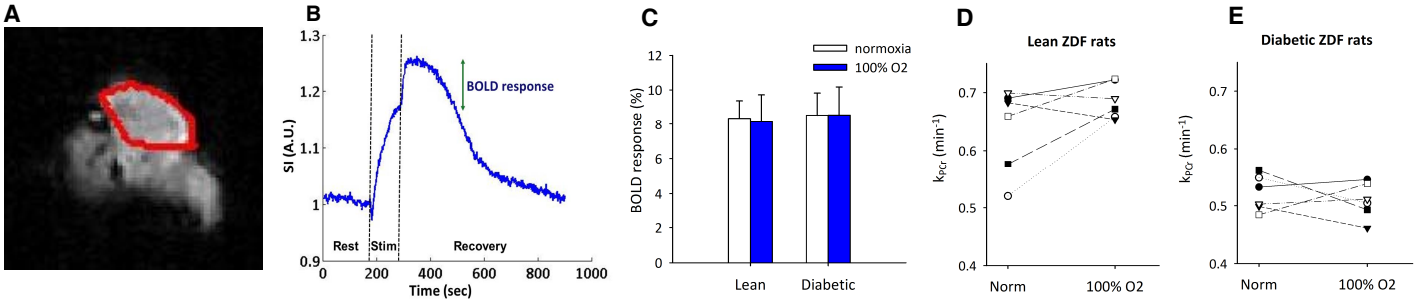

Figure 1. A) Example of a gradient echo EPI image with the ROI in TA muscle. B) Typical example of a BOLD measurement in TA muscle of a lean rat during stimulation and recovery. C) BOLD response after exercise in lean and diabetic rats. PCr recovery rate constants as determined from ^{31}P MRS during normoxic and hyperoxic conditions in D) lean and E) diabetic ZDF rats.

Results

O_2 saturation: In lean rats, O_2 saturation levels under normoxic conditions were 86 ± 1 % of those under hyperoxic conditions ($P < 0.01$, data not shown). Likewise, in diabetic rats, O_2 saturation levels under normoxic conditions amounted to 72 ± 4 % of the values under hyperoxic conditions ($P < 0.01$, data not shown).

BOLD imaging: The custom built force transducer was used to standardize the force output of the electrical stimulation, in order to be able to the compare the BOLD responses after muscle stimulation (Figure 1B). The BOLD response did not differ between lean and diabetic rats or between normoxic and hyperoxic conditions (Figure 1C).

^{31}P MRS: PCr recovery rate constants were 25% lower in diabetic compared with lean rats (0.52 ± 0.03 vs 0.66 ± 0.06 min^{-1}, $P < 0.01$). However, PCr recovery rate constants measured under normoxic and hyperoxic conditions did not significantly differ (Figure 1D,E) in both lean (0.64 ± 0.07 vs 0.68 ± 0.03 min^{-1}, $P = 0.17$) and diabetic rats (0.52 ± 0.03 vs 0.51 ± 0.03 min^{-1}, $P = 0.50$).

Discussion and Conclusion

In this study, BOLD imaging of the skeletal muscle in rats was successfully implemented resulting in quantitative measurements of the BOLD response after muscle stimulation. The BOLD responses did not differ between lean and diabetic rats or between normoxic and hyperoxic conditions, which implies that local oxygenation changes were similar between groups. However, it should be noted that these measurements were normalized relative to the baseline values, so absolute differences in SI at baseline were not taken into account. While PCr recovery was significantly slower in muscle of diabetic rats compared with lean rats, no differences were observed in PCr recovery rates between normoxic and hyperoxic conditions in either group. These results suggest that in these animals PCr recovery is not limited by oxygen delivery and that the reduced rate of PCr recovery in diabetic animals represents a decreased muscle mitochondrial content and/or impaired intrinsic mitochondrial function.

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