Increased glycolysis flux helps to maintain energy homeostasis in muscle of diabetic rats with mitochondrial dysfunction

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Target audience: Researchers interested in skeletal muscle energy metabolism

Introduction

Skeletal muscle mitochondrial dysfunction has been implicated in the pathogenesis of type 2 diabetes [1, 2]. When mitochondrial function is impaired, the cellular capacity for oxidative ATP synthesis is decreased. In addition to oxidative ATP production in the mitochondria, ATP is also produced by glycolysis in the cytosol. Both the glycolytic and mitochondrial metabolic pathways are internally regulated to maintain cellular energy homeostasis, but also to balance the mitochondrial versus glycolytic ATP synthesis fluxes thus ensuring metabolic flexibility [3]. We hypothesized that as a result of these regulatory mechanisms glycolytic ATP flux is increased in type 2 diabetic skeletal muscle to compensate for the impaired mitochondrial function. Alternatively, if compensation by glycolysis does not occur, the total ATP production capacity of the cell will be lower, which we expect will result in an earlier onset of muscle fatigue during exercise. In this study we investigated the effects of mild and more severe mitochondrial impairments on muscle fatigue and glycolytic ATP synthesis flux during muscle stimulation in rats. Glycolytic flux was derived from intracellular pH dynamics measured by in vivo 31P magnetic resonance spectroscopy (MRS). Zucker diabetic fatty (ZDF) rats were used as a model of mild mitochondrial dysfunction, while ZDF rats treated with the complex I inhibitor metformin served as a model of more severe mitochondrial dysfunction.

Materials and Methods

14-week old lean, healthy fa/+ (n = 8), obese, diabetic fa/fa (n = 8) and metformin-treated (300 mg/kg/day for 14 days) obese, diabetic fa/fa (n = 8) ZDF rats were used for the in vivo assessment of muscle mitochondrial oxidative capacity, glycolytic flux and muscle fatigue. 31P MRS was performed on the tibialis anterior (TA) muscle using a 6.3 T horizontal Bruker MR scanner, a circular ¹H surface coil (Ø 40 mm) for shimming purposes and an ellipsoid ³¹P surface coil (10/18 mm). ³¹P MR spectra (Figure 1A) were acquired using an adiabatic BIR4 pulse with a 90° flip angle. A fully relaxed spectrum (TR = 20 s, 32 averages) was measured at rest, followed by a time series of ³¹P spectra (TR = 5 s, 4 averages) before, during and after muscle contractions. Muscle contractions were induced by electrical stimulation of the TA, via subcutaneously implanted electrodes. The stimulation protocol consisted of 1 pulse train of 10 pulses applied once every 1.67 s for 3 min. The force produced by the TA during contraction was measured using a custom built force transducer. A read-out of the applied force was used to determine muscle fatigue during the 3 min stimulation protocol and was normalized relative to the maximal contractile force at the start of the protocol. The time-tension integral (TTI) was determined as the area under the contraction curve in the force-time diagram. Recovery was followed for 15 minutes. ³¹P MR spectra were fitted in the time domain using AMARES in the jMRUI software package. The recovery of phosphocreatine (PCr) was fitted to a mono-exponential function yielding the PCr recovery rate constant, krrec. Intracellular pH was calculated from the chemical shift difference between the P3, PCr resonances and used as non-invasive assessment of muscle mitochondrial oxidative capacity, glycolytic flux and muscle fatigue.

Results

Resting PCr, αP, ADP levels and pH did not differ between groups. PCr recovery rate constants were 30% lower in untreated diabetic rats and 50% lower in metformin-treated diabetic rats compared with the lean animals (Figure 1B). The relative reduction in peak force production during the 3 min of stimulation did not significantly differ between groups, indicating that muscle fatigue was similar in all groups (results not shown). However the time-tension integral was higher in both diabetic animal groups compared with the lean rats (Figure 1C). This is attributed to a slower relaxation after contraction of the muscle as a result of a decreased ATP supply. During stimulation, especially in the metformin-treated diabetic animals, the pH decline appeared to be faster than in controls (Figure 1D). Furthermore the end-exercise pH was found to be significantly lower in both untreated (6.44 ± 0.08, P = 0.02) and metformin-treated (6.34 ± 0.08, P < 0.001) diabetic rats compared with the lean rats (6.55 ± 0.06). The faster decrease in pH can be interpreted as a higher glycolytic flux in the animals with a reduced mitochondrial capacity. Additional analysis of the data by a detailed biophysical model of skeletal muscle energy metabolism will be performed to calculate mitochondrial and glycolytic contributions to ATP synthesis flux during muscle stimulation and to establish if the decreased mitochondrial capacity is indeed fully compensated for by increased glycolytic flux.

Discussion and Conclusion

It was demonstrated that untreated and metformin-treated diabetic rats have a lower muscle mitochondrial oxidative capacity and a faster decrease of muscle pH during muscle stimulation when compared with healthy controls. The latter implies that in both diabetic rat models glycolytic flux during muscle contractions was higher than in controls. Consequently, impairments in mitochondrial function of the skeletal muscle may actually increase the cellular demand for glucose, which could explain the therapeutic action of anti-diabetic drugs like metformin that are known to limit mitochondrial function. The mitochondrial impairments in untreated and metformin-treated diabetic rats were associated with a delayed relaxation of the skeletal muscle, which led to an increase in the time-tension integral during muscle contractions. The fact that ATP is consumed when Ca²⁺ ions are pumped back into the sarcoplasmic reticulum during muscle relaxation likely accounts for this observation.

References


Figure 1. A) Typical example of a ³¹P MR spectrum from TA muscle of a fa/+ rat. Peak annotations: P3, inorganic phosphate; PCr, phosphocreatine; γ, α, β, three phosphate groups of ATP. B) PCr recovery rate constants as determined from ³¹P MRS. C) Time-tension integral (TTI) determined from the force output during stimulation. D) Plot of average pH drop during exercise. Solid black lines represent linear fits through the pH data. fa/+, fa/fa and faM represent the lean, diabetic and the metformin-treated diabetic ZDF rats, respectively.* P<0.01, ** P<0.001 relative to fa/+ rats.