Metformin severely impairs in vivo muscle oxidative capacity in a rat model of type 2 diabetes

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Introduction

Metformin is a widely prescribed drug used for the treatment of type 2 diabetes. Its therapeutic action is primarily based on improving insulin sensitivity in the liver, thereby effectively inhibiting gluconeogenesis [1]. In addition, it has been shown that metformin improves insulin sensitivity of skeletal muscle resulting in enhanced peripheral glucose utilization, but the underlying mechanism still remains unclear. Previous studies have demonstrated that metformin specifically inhibits complex I of the mitochondrial respiratory system [2,3]. This seems counterproductive since impaired muscle mitochondrial function has been linked to the pathogenesis of type 2 diabetes [4,5]. However, it is not known whether and to which extend metformin affects muscle mitochondria in vivo. In this study we investigated the effects of metformin on in vivo and in vitro skeletal muscle mitochondrial function in Zucker diabetic fatty (ZDF) rats using 31P magnetic resonance spectroscopy (MRS) and high-resolution respirometry, respectively.

Materials and Methods

12-week old healthy fa/+ (317.5 ± 15.5 g) and diabetic fa/fa (358.8 ± 24.1 g) (i.e. ZDF) rats were treated with either metformin (300 mg/kg body weight/day) dissolved in 1 ml water (n = 6) or 1 ml water as a control (n = 6) for 15 days by oral gavage. At day 14, in vivo 31P MRS was performed on the tibialis anterior (TA) muscle using a 6.3 T horizontal Bruker MR scanner, an ellipsoid 31P surface coil (10/18 mm) and a circular 1H surface coil (240 mm; for shimming). 31P MR spectra (Figure 1A) were acquired using an adiabatic B1 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 a series of stimulation pulses, applied every second, for a duration of 2 min. Recovery was followed for 10 minutes. 31P MR spectra were fit in the time domain using AMARES in the jMRUI software package (jMRUI v2.1). The recovery of PCr was fitted to a mono-exponential function yielding the PCr recovery rate constant, k_{PCr}. One day after in vivo MRS, the animals were killed and TA muscles were excised for in vitro high-resolution respirometry measurements using a 2-channel high-resolution Oroboros oxygraph-2k (Oroboros, Innsbruck, Austria) at 37 °C. Mitochondria were isolated from one TA muscle and oxygen flux was measured using either pyruvate plus malate (for assessment of complex I-supported respiration) or succinate (complex II-supported respiration) plus rotenone (a complex I inhibitor) as a substrate. An ADP-regenerating system consisting of glucose (12.5 mM) and ATP (1 mM) was used to maintain steady-state maximal ADP-stimulated oxygen consumption rate (OXPHOS = classical State 3). 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.

Results

Body weight: Metformin treatment had no effect on body weight in fa/+ rats (340 ± 22 and 344 ± 11 g for untreated and metformin-treated fa/+ rats, respectively) or in fa/fa rats (388 ± 41 and 370 ± 18 g for untreated and metformin-treated fa/fa rats, respectively).

31P MRS results: PCr recovery rate constants were 25% lower in untreated diabetic fa/fa rats compared with untreated healthy fa/+ rats (Figure 1B). Metformin treatment decreased PCr recovery rates by two-fold in both healthy fa/+ and diabetic fa/fa rats (Figure 1B).

High-resolution respirometry: Complex I- (Figure 1C) and complex II-supported (Figure 1D) oxygen consumption rates in the OXPHOS state were similar in mitochondria isolated from TA muscle of untreated diabetic fa/fa and untreated healthy fa/+ rats. Irrespective of oxidizable substrate used, there was no effect of metformin treatment on OXPHOS oxygen consumption rates in TA mitochondria from the healthy fa/+ group. In contrast, complex I- and complex II-supported oxygen consumption rates in the OXPHOS state in mitochondria isolated from TA of metformin-treated diabetic fa/fa rats were 33% and 25% lower, respectively, compared with untreated diabetic fa/fa rats.

Discussion and Conclusion

Two weeks of metformin treatment led to a severe impairment of in vivo skeletal muscle oxidative capacity in TA of both diabetic fa/fa and healthy fa/+ rats. Based on the high-resolution respirometry data, the observation in diabetic fa/fa rats, but not in healthy fa/+ rats, could at least partially be explained by the inhibitory effect of metformin on mitochondrial complex I and II. The discrepancy between the effect of metformin treatment on in vitro mitochondrial function in skeletal muscle of diabetic fa/fa and healthy fa/+ rats suggests that disease progression may have caused subtle changes in e.g. the expression level(s) of metformin target(s) in skeletal muscle mitochondria of diabetic fa/fa rats, rendering them more sensitive to the inhibitory effect of metformin compared to healthy controls. In conclusion, we showed that metformin treatment severely impaired in vivo skeletal muscle oxidative capacity in rats, but this effect could not be fully attributed to inhibition of mitochondrial complex I. Since an impairment of mitochondrial function has been implicated in the pathogenesis of type 2 diabetes, our results suggest that the use of metformin to treat type 2 diabetes might need to be reevaluated.

References