Skeletal muscle mitochondrial function and intramyocellular lipids in the diabetic ZDF rat: a longitudinal study using *in vivo* ^{31}P and ^{1}H MRS

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Introduction

Recently skeletal muscle mitochondrial dysfunction has been related to insulin resistance and type 2 diabetes (T2D) in humans (1-3). Some authors suggest that impaired skeletal muscle mitochondrial function would be one of the first defects in the development of insulin resistance (2). Mitochondrial dysfunction would involve a reduced capacity to oxidize fatty acids, resulting in the accumulation of fatty acid intermediates which are known to interfere with the insulin signaling pathway. In situations of an oversupply (e.g. obesity) lipids could become per-oxidized and subsequently harmful for DNA and protein, thus inducing mitochondrial dysfunction (4). Data supporting these hypotheses are derived from cross-sectional studies, making it difficult to determine whether mitochondrial dysfunction is either a cause or a consequence of insulin resistance. For that reason we investigated both mitochondrial function and IMCL levels in a longitudinal study in the growing Zucker Diabetic Fatty (ZDF) rat. This rodent model is characterized by progressive loss of insulin sensitivity during maturation resulting in overt T2D in adulthood.

Materials and methods

In vivo NMR experiments were performed on ZDF fa/fa rats developing T2D (n = 10) and control fa/+ rats that remain normoglycemic (n = 11), all fed the same diet (PURINA 5008). At distinct time points during the pathogenesis of T2D (pre-diabetic: 6, diabetic: 12, and long-term diabetic: 18 weeks of age) mitochondrial function and IMCL were measured using in vivo MR spectroscopy (MRS)

All NMR experiments were performed on a 6.3 Tesla horizontal Bruker MR system. Skeletal muscle mitochondrial function was evaluated by means of dynamic ^{31}P MRS, measuring the post-exercise phosphocreatine (PCr) recovery kinetics of the tibialis anterior muscle (TA). Using a set-up with a combination of a circular ^{1}H surface coil and an ellipsoid ^{31}P surface coil, shimming was performed on the water signal and subsequently the acquisition of ^{31}P MR spectra was started. ^{31}P spectra were acquired applying an adiabatic pulse with a flip angle of 90 degrees. A fully relaxed spectrum (TR = 20 s, 32 averages) and a saturated spectrum (TR = 5 s, 128 averages) were measured at rest, followed by the acquisition of a time series of spectra (TR = 5 s, 4 averages) before, during and after the muscle contractions. Muscle contractions were induced by electrical stimulation, via acutely subcutaneously implanted platinum electrodes. The muscle exercise consisted of a series of stimulation pulses, applied every second, for a duration of 2 min. The stimulation pulse length was 100 ms, the stimulation frequency was 80 Hz and the voltage varied between 2 and 4 V.

Localized ^{1}H MRS was applied to measure IMCL levels in the TA as previously described (5). In short, 2 voxels of $2 \times 2 \times 2$ mm^3 were placed in the white (ventral) and red (dorsal) TA using an ellipsoid ^{1}H surface coil. Single-voxel localized ^{1}H MR spectra were acquired using the LASER sequence (TR = 1 s, TE = 16 ms, SWAMP water suppression, 512 averages). Unsuppressed water spectra (32 averages) were recorded from the same voxel and used as an internal reference.

Both ^{31}P and ^{1}H spectra were fitted in the time domain by using a nonlinear least squares algorithm (AMARES) in the jMRUI software package (6). For the time series, the PCr line width during recovery was constrained to the average PCr line width during recovery (excluding the first 5 data points), obtained from a prior, unconstrained fit. The recovery of PCr was fit to a mono-exponential function yielding a time constant, τ_{PCr}, indicative for skeletal muscle mitochondrial oxidative capacity (figure 1).

The data were analyzed statistically by applying a General Linear Model for repeated measurements using SPSS 14.0 (SPSS Inc, Chicago, IL, USA). Level of significance was set at $p < 0.05$. Data are presented as mean ± SD.

Results

The fa/fa animals were normoglycemic at 6 weeks of age, while they were diabetic at 12 and 18 weeks of age (fasting blood glucose = 5.3 ± 0.8, 17.5 ± 2.2 and 19.6 ± 1.8 mM, respectively). The fa/+ animals remained normoglycemic at all ages (fasting blood glucose = 4.3 ± 0.6, 5.2 ± 0.4 and 6.0 ± 0.5 mM, respectively). In the pre-diabetic state (6 weeks of age) the fa/fa animals showed higher IMCL levels in the white and red region of the TA compared to the fa/+ animals (figure 2). At 12 and 18 weeks of age, during overt T2D, the IMCL levels were more than 5 times higher in fa/fa than in fa/+ animals. The τ_{PCr} was identical at 6 weeks of age (figure 3). In both rat genotypes oxidative capacity decreased with maturation. At 12 weeks of age, the fa/fa animals appeared to have a lower oxidative capacity (i.e., a higher τ_{PCr}) than the fa/+ genotype. However, at 18 weeks of age, both genotypes showed again similar values for τ_{PCr}.

Discussion

The fasting blood glucose levels measured at the different ages confirmed that the fa/fa animals were pre-diabetic at 6 weeks of age and became diabetic thereafter, whereas the fa/+ rats remained normoglycemic during the entire study. The increased IMCL levels at 6 weeks of age and the decreased oxidative capacity at 12 weeks of age in the fa/fa rats, could point towards a lipid oversupply-induced quality loss of the mitochondria. The progressive nature of this process could explain that the lowest oxidative capacity was measured at 18 weeks, when the animals had been diabetic for the longest period. However, the control fa/+ animals showed a similar oxidative capacity at 18 weeks without increased IMCL levels suggesting that the disease only accelerated the natural aging process in the diabetic rats.

In conclusion, in this rat model the impact of T2D on skeletal muscle mitochondrial function appeared relatively small or at least disproportionate to the status of T2D. The present study also showed that in this genetic model of T2D, mitochondrial dysfunction is not the first aberration in the pathogenesis of T2D, since IMCL accumulation occurred before any difference in mitochondrial oxidative capacity was detected. Further experiments are warranted to identify the mechanisms underlying the decrease in mitochondrial oxidative capacity observed in the fa/fa animals at the age of 12 weeks.

References: (1). Petersen KF, *et al*. Science, 2003 (2). Petersen KF, *et al*. N Engl J Med, 2004 (3). Kelley DE, *et al*. Diabetes, 2002 (4). Schrauwen P, *et al*. Diabetes, 2004 (5). De Feyter HM, *et al*. Magn Reson Med, 2006 (6). Vanhamme L, *et al*. J Magn Reson., 1997

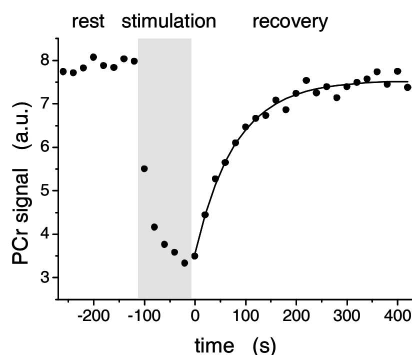

Figure 1. PCr signal during a rest-stimulation-recovery protocol. Time resolution = 20 s. A mono-exponential function (black line) was fit trough the actual data.

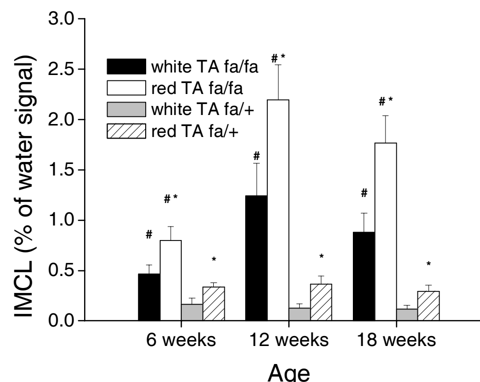

Figure 2. IMCL levels from white and red region of the TA muscle from the different genotypes, at different ages. * p < 0.05 vs. white TA from same genotype. # p < 0.05 vs. same muscle region from different genotype.

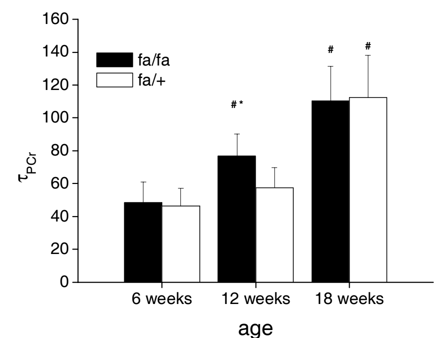

Figure 3. τ_{PCr} from different genotypes at different ages. * p < 0.05 vs. τ_{PCr} from fa/+ at the same age. # p < 0.05 vs. τ_{PCr} of previous time point (age) from same genotype.