Metabolic adaptations in skeletal muscle in the early stage of insulin resistance measured *in vivo* by ^{1}H and ^{31}P MRS

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Background & Aim

Current knowledge indicates that the pathology of insulin resistance involves metabolic alterations in several tissues, including skeletal muscle. Recent experiments have shown that mitochondrial oxidative phosphorylation and expression of genes involved in oxidative phosphorylation are reduced in muscle of insulin-resistant patients (1, 2). Decreased muscle fat oxidation can favor the accumulation of intramyocellular lipids (IMCL), which has been associated with reduced insulin sensitivity. Therefore, impairments of mitochondrial capacity might be a major risk factor predisposing individuals to develop insulin resistance. We investigated skeletal muscle oxidative capacity and IMCL content in a rat model of early, lifestyle-induced insulin resistance using *in vivo* ^{1}H and ^{31}P magnetic resonance spectroscopy (MRS).

Methods

Ten, 14 week old Wistar rats were put on a high fat diet (HFD, 45.7 energy% fat) for 2 weeks (HF) and a control group of 10 Wistar rats received normal chow (NC). Glucose tolerance was measured with an oral glucose tolerance test (OGTT) (1 g/kg body weight).

All MRS experiments were performed on a 6.3 Tesla horizontal Bruker MR system. Localized ^{1}H MRS was applied to measure IMCL levels in the tibialis anterior (TA) muscle using an ellipsoid ^{1}H surface coil (18/22 mm). Two voxels of 2x2x2 mm^3 were placed in the white (ventral) and red (dorsal) TA (TA1 and TA2, respectively). 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.

Skeletal muscle oxidative capacity was determined *in vivo* using dynamic ^{31}P MRS. ^{31}P MRS was applied using a combination of a circular ^{1}H surface coil (40 mm diameter) and an ellipsoid ^{31}P coil (10/18 mm) positioned over the TA. ^{31}P MR spectra were acquired applying an adiabatic excitation pulse with a flip angle of 90 degrees. A fully relaxed spectrum (TR = 20 s, 32 averages) and a partially 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 muscle contractions. Muscle contractions were induced by electrical stimulation of the TA, via 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.

Both ^{1}H and ^{31}P MR spectra (figure 1) were fitted in the time domain by using a nonlinear least squares algorithm (AMARES) in the jMRUI software package. IMCL was expressed as a percentage of the water signal, measured in the same voxel. For the ^{31}P 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}, which is a measure of the skeletal muscle mitochondrial oxidative capacity.

Data were analyzed statistically by applying two-tailed t-tests using Statgraphics Centurion XV.II (StatPoint Inc, Virginia, USA). Level of significance was set at $p < 0.05$. Data are presented as means ± SD.

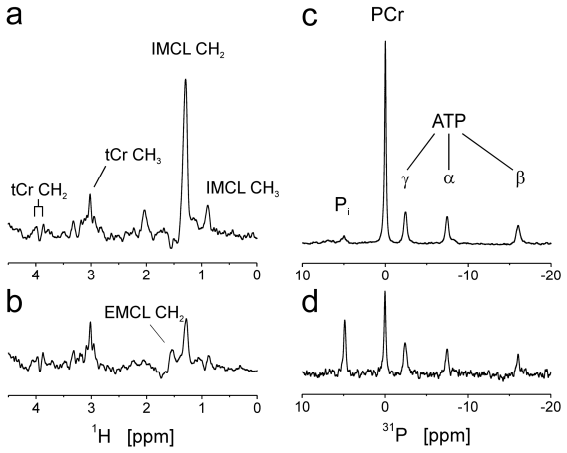
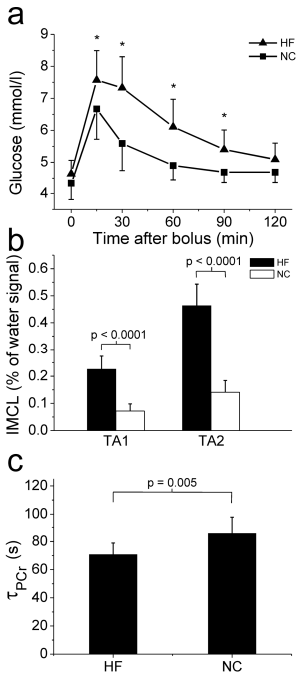


Figure 1. Water suppressed ^{1}H MR spectrum from TA2 in HF rat (a) and NC rat (b). Partially saturated ^{31}P MR spectrum at rest (c, nt = 128) and at the end of stimulation (d, nt = 4).



Results

At 16 weeks of age, the HF animals showed a significantly higher area under the curve for glucose during the OGTT compared to NC animals (12.25 ± 1.06 vs. 10.27 ± 0.79 mM·h, p = 0.0002) (figure 2a). Insulin levels are not determined yet, but both literature data and data from a pilot study showed increased insulin levels during the OGTT in rats after 2 weeks of HFD. These results indicate that after 2 weeks of HFD, rats have become insulin resistant.

For both groups, IMCL content in the dorsal region of the TA (TA2) was about 2 times higher than in the ventral region (TA1) (figure 2b), which is in agreement with previous results (3). IMCL levels were about 3 times higher in HF compared to NC rats for both voxels (TA1: 0.226 ± 0.048 % vs. 0.072 ± 0.026 %, $p < 0.0001$ and TA2: 0.463 ± 0.080 % vs. 0.141 ± 0.043 %, $p < 0.0001$).

τ_{PCr} was shorter in the animals on HFD compared to the animals on normal chow (70.69 ± 8.31 s and 85.71 ± 12.14 s, respectively, p = 0.005) (figure 2c). The end-exercise pH and PCr concentration did not differ between groups (7.04 ± 0.05 and 7.01 ± 0.05, p = 0.23 and 20.39 ± 2.39 mM and 21.43 ± 2.73 mM, p = 0.37, respectively for the HF and NC groups).

Discussion & Conclusion

We found that, in a rat model of early, HFD-induced insulin resistance, IMCL levels were elevated in the TA muscle. Interestingly, *in vivo* skeletal muscle oxidative capacity was higher in the insulin resistant rats compared to control rats. This can be explained as an adaptation to overfeeding of the mitochondria. Persistent overfeeding might have more detrimental effects in the long term. As part of a longitudinal study, we will study the same animals also during a more advanced stage of insulin resistance.

In conclusion, mitochondrial dysfunction does not play a role in the early stage of insulin resistance in a validated animal model of lifestyle-induced insulin resistance.

References: (1) Patti ME et al. Proc Natl Acad Sci USA **100** 8466-8471; 2003. (2) Petersen KF et al. N Eng J Med **350** 664-671; 2004. (3) De Feyter HM et al. Magn Reson Med **56** 19-25; 2006.

*Figure 2. a) Blood glucose concentrations after a glucose load in HF and NC rats, * p < 0.05, b) IMCL levels in TA1 and TA2 in HF and NC rats, c) τ_{PCr} in HF and NC rats.*