In vivo assessment of the effects of pioglitazone on muscle oxidative capacity and intramyocellular lipid content in diabetic rats using $^{31}$P and $^1$H MRS

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Introduction
Skeletal muscle mitochondrial dysfunction and excessive accumulation of intramyocellular lipids (IMCL) have been implicated in the development of insulin resistance [1-2]. Thiazolidinediones (TZDs), such as pioglitazone and rosiglitazone, are insulin-sensitizing drugs that are commonly used to treat patients with type 2 diabetes. TZDs act on the PPAR-γ receptor thereby stimulating the expression of genes involved in fat oxidation and mitochondrial biogenesis in adipocytes [3] and promoting adipocyte maturation [4]. This leads to a diversion of lipids from ectopic sites, such as skeletal muscle, into subcutaneous adipose tissue [3]. In addition, it has been proposed that TZDs could potentially improve mitochondrial function in skeletal muscle [5]. However, a recent study in patients with type 2 diabetes showed that rosiglitazone improves insulin sensitivity without altering muscle mitochondrial function or IMCL content [5]. The aim of the present study was to investigate if the insulin-sensitizing effect of pioglitazone is accompanied by improvement of in vivo skeletal muscle mitochondrial function and normalization of IMCL levels in a rat model of type 2 diabetes using $^{31}$P and $^1$H magnetic resonance spectroscopy (MRS), respectively.

Materials and Methods
12-week old non-diabetic fa/+(317.9 ± 20.3 g) and diabetic fa/fa (365.6 ± 26.3 g) Zucker diabetic fatty (ZDF) rats were treated with either pioglitazone (30 mg/kg body weight/day) dissolved in 1 ml water (n = 6) or 1 ml water for 14 days by oral gavage. After 13 days of treatment a glucose tolerance test (OGTT) was performed after a 4 h fast. Plasma glucose concentrations were determined using an automatic glucometer (Freestyle, Abbott, IL, USA) and the area under the glucose curve (AUC) was calculated. At day 14, in vivo $^1$H and $^{31}$P MRS were performed on the tibialis anterior (TA) muscle using a 6.3 T horizontal Bruker MR scanner, a circular $^1$H water surface coil (20-40 mm) and an ellipsoid $^{31}$P surface coil (10/18 mm). Localized $^1$H MR spectra (Figure 1A) were acquired using PRESS (TR = 1.5 s, TE = 9.4 ms, VAPOR water suppression, 256 averages) in a voxel of 3 x 3 x 3 mm$^3$ in the dorsal part of the TA close to the tibia bone. $^1$H MR spectra (Figure 1C) 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 $^{31}$P 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. Both $^1$H and $^{31}$P MR spectra were fitted in the time domain using AMARES in the jMRUI software package. IMCL content was expressed as a percentage of the water signal obtained from a spectrum without water suppression (16 averages) recorded in the same voxel. The recovery of phosphocreatine (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.

Results
Body Weight: Pioglitazone treatment did not affect body weight in fa/+ rats (340 ± 22 and 355 ± 25 g for untreated and pioglitazone-treated fa/+ rats, respectively) or in fa/fa rats (388 ± 41 and 412 ± 30 g for untreated and pioglitazone-treated fa/fa rats, respectively).

Plasma glucose: Fasting plasma glucose levels were 3-fold higher in untreated fa/fa rats compared with untreated fa/+ rats (17.0 ± 0.9 and 4.7 ± 0.3 mM, respectively). Treatment with pioglitazone significantly lowered fasting plasma glucose concentrations in the fa/fa group (12.6 ± 4.7 mM), but had no effect on fasting plasma glucose in fa/+ rats (4.5 ± 0.4 mM). Glucose tolerance in fa/fa rats was not significantly improved by pioglitazone treatment (AUC$_{G}$: 40.0 ± 2.0 and 32.7 ± 10.8 mM$\cdot$h for untreated and pioglitazone-treated fa/fa rats, respectively).

$^1$H MRS: IMCL content was 7-fold higher in untreated fa/fa rats compared with fa/+ rats (Figure 1B). Pioglitazone treatment lowered IMCL content in fa/fa rats by 50%, but after treatment IMCL levels in fa/fa rats were still 3-fold higher than in fa/+ rats. In fa/+ rats, pioglitazone had no effect on IMCL levels.

$^{31}$P MRS: Resting PCr, P$_{i}$, and ADP levels and pH did not differ between groups. PCr recovery rate constants were significantly lower in untreated fa/fa rats compared with fa/+ rats (Figure 1D). Treatment with pioglitazone normalized the rate of PCr recovery in fa/fa rats to the values of fa/+ rats, whereas it had no effect in fa/+ rats.

Discussion and Conclusion
In diabetic fa/fa rats, IMCL content was higher and in vivo muscle oxidative capacity was lower than in non-diabetic fa/+ rats. Two weeks of treatment with the PPAR-γ agonist pioglitazone lowered fasting plasma glucose levels in diabetic fa/fa rats. This was paralleled by a decrease in IMCL content and an increase in vivo muscle oxidative capacity. These results confirm the association between type 2 diabetes, reduced mitochondrial oxidative capacity in skeletal muscle and excessive accumulation of IMCL. Moreover, it suggests that the insulin-sensitizing effect of pioglitazone is brought about by improvement of muscle mitochondrial function and partial normalization of IMCL. Further studies are needed to establish whether pioglitazone primarily lowers IMCL content leading to increased mitochondrial oxidative capacity or if pioglitazone improves muscle mitochondrial function resulting in a decrease in IMCL content.

References

Figure 1. A) Typical example of a $^1$H MR spectrum from TA muscle of an untreated fa/+ rat. Peak annotations: tCr, total creatine; EMCL and IMCL, extramyocellular and intramyocellular lipids. B) IMCL content as determined from $^1$H MRS spectra. C) Typical example of a $^{31}$P MR spectrum from TA muscle of an untreated fa/+ rat. D) PCr recovery rate constants as determined from $^{31}$P MRS. * p<0.01 relative to fa/+ rats ** p<0.001 relative to fa/+ rats, * P<0.01 relative to untreated rats.