Effects of diet manipulation on muscle mitochodrial activity as assessed in rat by 31P saturation transfer

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Abstract

In-vivo ^{31}P saturation transfer MRS was used to support the existence of measurable changes in mitochondrial function of rat muscles in response to diet manipulation. The ATP synthesis rate decreased up to 50% within 24 hr of raising fat content in the diet from 12% to 60% of the caloric intake, but eventually returned to normal levels after ~3 weeks on the HF regimen, most likely to prevent further fat accumulation, as demonstrated by parallel measurements of intramyocellular lipids. It is only beyond one month on the HF diet that results consistently showed 30 to 50% lower ATP synthesis rates.

Introduction

Recent clinical data obtained from both elderly patients [1] and offsprings of parents with type 2 diabetes [2] have shown that subtle and early defects in mitochondrial oxidative capacity can lead to an accumulation of intracellular fatty acyl CoA and harmful metabolites that disrupt insulin signalling. Given that skeletal muscle is a major site of insulin resistance, therapeutic strategies aimed at increasing fatty acid oxidation in the muscle represent promising targets for future treatments of type 2 diabetes. Yet, the success of future profiling studies is bound to the use of a non-invasive marker of mitochondrial activity. The ^{31}P magnetization transfer technique offers the unique possibility to determine, *in vivo*, certain reaction rates without disturbing the chemical equilibrium. The objective of this study was to ascertain the relationship between mitochondrial function and insulin resistance upon diet manipulations in rat muscle. Muscle mitochondrial activity was determined through the measurement of the F_1F_0 ATP synthase flux, the terminal step in the oxidative phosphorylation process. To this end, ^{31}P saturation transfer was used to measure a decrease in the inorganic phosphate (Pi) signal due to the magnetization exchange between ATPγ and Pi upon saturation of the ATPγ signal *in vivo*.

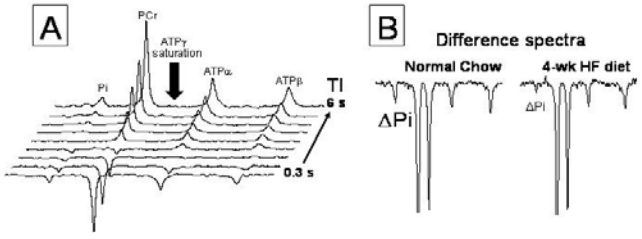
Methods

Measurements were carried out in 3 to 6-month old rats fed either with a normal chow diet or a 60% fat-enriched (HF) diet, depending on the experiment. All NMR data were obtained under 2% isoflurane anesthesia, using a Bruker Biospec 7T/30cm instrument equipped with a 20-cm i.d. gradient insert. A ^{1}H/^{31}P double-tuned surface coil with a 2.5 cm i.d. was used to collect signal from the lower leg of the rat. The measurement of the ATP synthesis rate was systematically combined with the measurement of intramyocellular lipid (IMCL) levels during the same NMR session. The saturation transfer experiment requires that two spectra be acquired, one with and one without (control spectrum) steady-state saturation of the γ-ATP peak. Selective irradiation of the γ-ATP resonance was achieved using a continuous wave pulse applied at a low power for a relatively long time (i.e. mixing period of 5.9 s). For the control spectrum, the saturation pulse was placed at a frequency offset equidistant downfield of Pi. Each acquisition utilized 128 averages, with a repetition time of 6 s, leading to a total acquisition time of 13 min/spectrum. The ratio of the resulting magnetization (M_z) to the equilibrium magnetization (M_0) in the absence of γ-ATP saturation was given by the equation: $M_z/M_0=1/(1+kT_1)$, where k is the rate constant describing the loss of magnetization from Pi, and T_1 is the longitudinal (spin-lattice) relaxation time for the Pi nucleus. For accurate measurements, the spin lattice relaxation time was also evaluated (i.e. observed T_{1obs}) on an individual basis in the presence of continuous γ-ATP saturation (Figure 1A). While applying an inversion recovery (IR) pulse during the mixing period, the apparent T_{1obs} is related to T_1 by the following equation: $1/T_{1obs}=1/T_1+k$. The inversion pulse consisted in a 2s sech pulse, applied at 6 inversion delays (299 to 5999 ms) from the 90° detection pulse to invert all Pi spins during the γ-ATP saturation. Each IR spectrum was averaged 32 times, leading to a total experimental time of ~20 min. Individual T_{1obs} values were calculated using a nonlinear least-square fitting method based on the following equation: $M=M_o[1-2e^{(-TI/T1obs)}]$. The decrease in P_i signal (ΔM) used to determine the kinetics of ATP_{ase} activity (i.e. the rate constant k) was computed by simultaneously solving the various equations previously described, yielding the following equation: $k=1/T_{1obs}\times\Delta M/M_0$, where $\Delta M=M_0-M_z$. The unidirectional ATP synthesis flux was then calculated by multiplying the constant k by the Pi concentration extrapolated from the baseline NMR spectrum and [ATP] measurements obtained biochemically from calf muscles of 35 normal rats. For IMCL measurements, proton spectra were obtained from the *tibialis anterior* (*TA*) muscle of the left leg, using a PRESS sequence (2x2x2mm^3 voxel, TR/TE=2s/20ms, 256 averages) with water CHESS suppression. Prior to this, scout images were acquired to carefully position the volume of interest. Peak areas for total creatine (tCr: 3.0ppm, internal reference), EMCL (1.5ppm) and IMCL (1.3ppm) were quantified using a line fitting procedure. All data are presented as means±SE.

Results

Variations in the ATP synthesis rate measured during test-retest studies did not exceed 10%. Furthermore, inter-group differences for the same variable did not seem to be hindered by anaesthesia, while the dynamic range was estimated from ~100 to 300 nmol/g/sec. Results showed ATP synthesis rates may decrease up to 50% within 24 hr ($p<0.05$) of raising the fat content in the diet to 60% of the caloric intake. ATP synthesis rates eventually returned to normal levels after 1 week on the HF regimen, seemingly to prevent further accumulation of IMCL (i.e. IMCL/tCr ratio of ~3.0, $p<0.05$ vs. baseline). Interestingly, switching back to a normal chow diet after ~2 weeks on HF regimen was followed by rapid normalization of IMCL, returning to baseline values after only 2 days. At this point, ATP synthesis rates also transiently decreased by ~30% ($p<0.05$), indicative of a temporal disconnect between mitochondrial activity and lipid utilization. It is only beyond one month on the HF diet that results consistently showed 30 to 50% lower ATP synthesis rates vs. rats on normal chow diet ($p<0.05$), as well as greater IMCL contents (i.e. IMCL/tCr ratio of ~6.0, $p<0.05$ vs. 1-month) (Figure 1B). Metabonomic analysis of muscle extracts indicated the HF diet had a negative impact on muscle energy metabolism. In addition, the experiment showed that the HF diet-induced reduction in muscle ATP turnover was not only correlated to increased IMCL levels, but glucose intolerance as well. Whether this is a causative relationship still needs to be investigated.

Figure 1 – Illustration of typical inversion recovery spectra obtained from the rat muscle for T_{1obs} determination of Pi while ATPγ was saturated (A), and ^{31}P magnetization transfer difference spectra obtained from two age-matched rats after 4 weeks on two different diet regimens (B)



Discussion

These results showed muscle mitochondrial activity is measurable *in vivo* in the anaesthetized rat. ATP synthesis flux reduction up to 50% can be repeatedly detected in rats fed for over 4 weeks with a high-fat diet. Such a defect was well correlated with an accumulation of IMCL. Whether at this stage, IMCL accumulation in the HF fed rats resulted from an inherent defect in oxidative phosphorylations or inhibition of mitochondrial capacity in "burning" fat by the lipid intermediates themselves remains to be determined. With respect to data obtained within days after switching back to a normal fat diet, the fact that mitochondrial activity temporally disconnects itself from IMCL levels would indicate that substrate oxidation may drop through a decreased mitochondrial capacity to use lipids that are at such point in time scarce as metabolic fuels.

References

1. Petersen *et al* Science 300:1140, 2003 2. Morino *et al* JCI 115: 3587, 2005