Insulin-stimulated mitochondrial ATP synthesis is impaired in rat muscle by fat-enriched diet

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Introduction

Recent data obtained from the offspring of patients with type II diabetes suggested that an inherited defect in mitochondrial activity, assessed through *in vivo* measurement of ATP synthesis rate, is associated with intramyocellular lipid (IMCL) accumulation and may underlie the development of insulin resistance in muscle [1]. Although the causative nature of this relationship has not yet been established, modulating circulating lipid levels, either acutely (e.g. through lipid infusion in humans, [2]) or chronically (e.g. by a high-fat diet regimen, [3]) has shown that increased free fatty acid levels lead to similar results, both in terms of depressed ATP synthesis rates and elevated IMCL contents. Recent data have also shown that raising insulin to physiological levels induces up to 90% increase in muscle ATP synthesis rate of normal individuals [1, 2]. However in insulin-resistant patients, the stimulatory effect of insulin was diminished [1]. To our knowledge, no such data has been demonstrated in pre-clinical models. To this end, the objective of this study was to ascertain the *in vivo* relationship between mitochondrial function, IMCL and insulin resistance in rat muscle. Muscle mitochondrial activity was determined using ^{31}P saturation transfer, as recently published [3], measured before and during the steady state of a euglycemic-hyperinsulinemic clamp in normal and diet induced obese (DIO) rats.

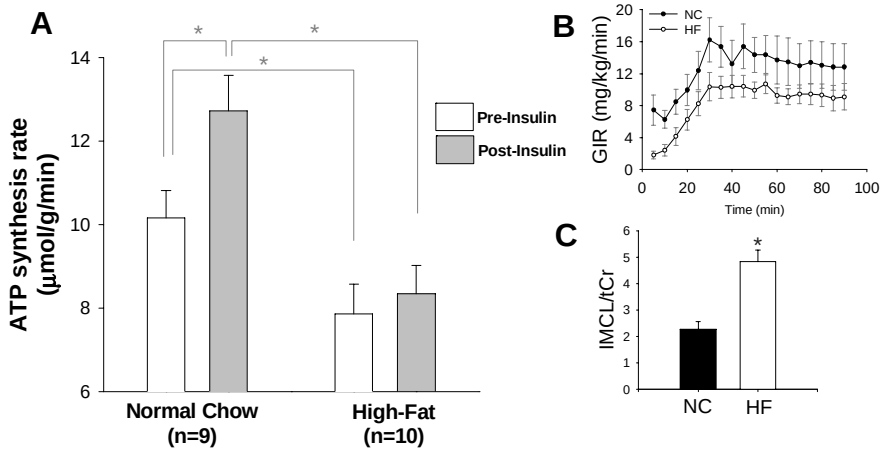
Methods

Measurements were carried out in 12 to 14-week old rats fed with either a normal chow diet (n=9) or a 60% fat-enriched (HF) diet (n=10) that were fasted the evening prior to the experiment. One hour before imaging, the rat was anesthetized and the jugular vein and carotid artery were cannulated. All NMR data were obtained under 1-2% 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. Measurement of the ATP synthesis rate was systematically combined with the measurement of IMCL levels and the longitudinal (spin-lattice) relaxation time, T_1, for the Pi nucleus during the same NMR session. These measurements, including the saturation transfer experiment which required the acquisition of two spectra, one with and one without (control spectrum) steady-state saturation of the γ-ATP peak were carried out as recently described [3]. Following surgery, the rat was laid prone on a supportive bed and the cannula to the jugular vein was connected to two pumps, one containing 30% glucose and the other with 0.8 U/mL Humulin in 0.1% BSA saline and 20 U/ml heparin. Before beginning the clamp, the basal ATP synthesis rate and related T_1 were measured by ^{31}P NMR. Following these measurements, the pumps were activated. For the first 10 minutes, a bolus of 32 mU/kg/min insulin was administered, and, for the remainder of the clamp, the infusion rate of insulin was held constant at 16 mU/kg/min. Blood was collected via the cannulated artery at baseline and every 5 minutes during the insulin infusion to monitor blood glucose levels and adjust the glucose infusion rate to achieve a steady state blood glucose of 140 mg/dl. While reaching the steady state, IMCL was measured by localized ^{1}H MRS. Once the metabolic steady state was reached, ATP synthesis rates were measured once more. After 90 minutes infusion, the liver and TA muscle were excised and flash frozen for later analysis. All data are presented as mean±SEM.

Results

HF rats showed significantly elevated levels of IMCL/tCr (4.84±0.44 vs chow, 2.54±0.27), a well known early marker for impaired insulin sensitivity. Basal glucose levels were 10% higher (ns) in HF fed rats. Animals reached a steady glucose infusion rate after 30 min, with variability not exceeding 2% over the last ½ hour of the clamp when glucose infusion rate (GIR) was calculated. GIR data also supported that DIO rats were less insulin sensitive than age-matched chow fed rats (GIR 45-50% less in HF fed rats, $p<0.05$) and GIR values were well correlated with measured IMCL levels ($r=0.77$, $p<0.05$). ATP synthesis rates as measured in normal chow rats under baseline were 30% ($p<0.05$) higher than in HF rats. In addition, basal rates of muscle ATP synthesis were associated with GIR ($r=0.50$ $p<0.05$). Chow diet control animals also showed significant increase in mitochondrial phosphorylation (20%) activity upon insulin stimulation, while age matched DIO rats showed no substantial change (Figure 1). Finally, the correlation between GIR and ATP synthesis rates appeared to be stronger upon insulin stimulation ($r=0.66$, $p<0.005$).

Figure 1 – A: ATP synthesis rates before and during the clamp steady-state. B: GIR data for each diet group C: IMCL/tCr ratio showing significant elevation ($p<0.05$) after 8-10 wks on HF diet.



Discussion

These data confirm findings shown clinically in insulin-resistant patients [1]. Specifically, ATP synthesis rates in DIO rats exhibit a blunted response to insulin stimulation, most likely indicative of a diminished mitochondrial activity. While increased lipid availability has long been associated with impaired insulin signaling, it has also been suggested that a reduction in PGC-1alpha, which is involved in mitochondrial biogenesis, may result from chronic consumption of excess dietary fat. These results also indicate the importance of the insulin challenge, not only as a state more relevant than the fasting condition for study of mitochondrial function, but also one that improves both the dynamic range and sensitivity of the ATP readout. This study establishes this method as a fully translatable approach for testing treatments aimed at improving mitochondrial activity in skeletal muscles, which account for ~80% of glucose disposal.

References

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