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Insulin-stimulated mitochondrial adenosine triphosphate synthesis is blunted in skeletal muscles of high-fat–fed rats

Brittany Yerby[a], Richard Deacon[b], Valerie Beaulieu[b], Jinsheng Liang[b], Jiaping Gao[b], Didier Laurent[a,*]

[a]*Global Imaging Group, Novartis Institutes for BioMedical Research, Inc, Cambridge, MA 02139, USA*
[b]*Diabetes and Metabolism, Novartis Institutes for BioMedical Research, Inc, Cambridge, MA 02139, USA*

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Abstract

Physiologic elevation of insulin levels induces a significant increase in muscle adenosine triphosphate (ATP) synthesis rate in normal individuals, indicative of an appropriate acceleration in mitochondrial activity. However, the stimulatory effect of insulin is diminished in insulin-resistant patients. In the absence of similar data from preclinical models, the present study investigated the inhibitory effects of increased dietary fat intake on insulin-stimulated ATP synthesis rates in rats. After being placed on a high-fat diet for 8 weeks (n = 10), diet-induced obese male Sprague-Dawley rats were tested against age-matched control rats (n = 9) on a normal chow diet. Muscle ATP synthase flux rates were measured under anesthesia by in vivo ^{31}P saturation transfer both before and during a euglycemic-hyperinsulinemic clamp. The glucose infusion rates observed during the clamp revealed impaired peripheral insulin sensitivity in the high-fat–fed rats when compared with the age-matched control rats. Under baseline conditions (ie, low insulin), the muscle ATP synthesis rates of high-fat–fed rats were approximately 30% lower ($P < .05$) than those in chow-fed rats. Moreover, chow-fed animals showed a significant increase (25%, $P < .05$ vs basal) in muscle ATP synthesis activity upon insulin stimulation, whereas high-fat–fed animals displayed no substantial change. These data demonstrated for the first time in a preclinical model that the insulin challenge not only facilitates an improvement in the dynamic range of ATP turnover measurement by ^{31}P saturation transfer between normal and insulin-resistant rats, but also mimics challenge that is relevant for pharmacologic studies on antidiabetic drugs aimed at improving mitochondrial function.

1. Introduction

Therapeutic strategies aimed at increasing fatty acid oxidation in the muscle present promising targets for future treatments of type 2 diabetes mellitus (T2DM). Recent data obtained from the offspring of patients with T2DM suggest that an inherited defect in mitochondrial activity, assessed through in vivo measurement of the adenosine triphosphate (ATP) synthesis rate, is associated with intramyocellular lipid (IMCL) accumulation and may underlie the development of insulin resistance in muscle [1,2]. Although the causative nature of this relationship has not yet been established, modulating circulating lipid levels, either acutely (eg, through lipid infusion in humans [3]) or chronically (eg, by a high-fat diet regimen [4]), has shown that increased free fatty acid levels yield similar results both in terms of depressed ATP synthesis rates and elevated IMCL contents. Recent data have also shown that physiologically raising insulin levels may induce an up to 90% increase in the muscle ATP synthesis rate of normal individuals [2,3], whereas this stimulatory effect of insulin was moderated in insulin-resistant patients [2].

In view of such results, it has been suggested that a therapy aimed at improving the functionality of the mitochondrial system would increase the uptake of glucose into the muscle (insulin response) [5]. The need for highly sensitive biomarkers addressing mitochondrial activity has therefore led to the hypothesis that the measurement of ATP production may be more appropriate under insulin challenge conditions. To our knowledge, no such data have been demonstrated in preclinical models. To this end, the objective of this study was to ascertain an in vivo relationship between mitochondrial function, IMCL, and insulin resistance in rat muscle upon insulin stimulation. Muscle mitochondrial activity was determined using

* Corresponding author. Tel.: +1 617 871 3821; fax: +1 617 871 4097.
E-mail address: didier.laurent@pharma.novartis.com (D. Laurent).

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^{31}P saturation transfer, as recently published [4], measured before and during the steady state of a euglycemic-hyperinsulinemic clamp in anesthetized normal and diet-induced obese (DIO) rats.

2. Methods

2.1. Animals

Adult (12-14 weeks old) male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed with a 12-hour light/dark cycle and had free access to study diet and water. All experimental procedures were carried out in compliance with the guidelines and with the approval of the Novartis Institutional Animal Care and Use Committee.

2.2. Determination of optimal insulin infusate concentration

For this preliminary study, fifteen 12-week–old rats on normal chow diet (5053, 13% calories from fat; PicoLab Diet 20, Richmond, IN) were clamped using different concentrations of insulin infusate to determine which infusion rate would yield the highest glucose infusion rate (GIR) to be used in later in vivo metabolic studies. Animals were arbitrarily divided into 5 dose groups (2, 4, 8, 16, and 32 mU kg^{-1} min^{-1} insulin; Eli Lilly, Indianapolis, IN; n = 3 per group), and 4 to 6 rats were clamped each day over a 3-day period. Rats were fasted at 5:00 PM for the following day's study. On the day of the clamp, animals were anesthetized with 1% to 2% isoflurane (Baxter Healthcare, Deerfield, IL) for the cannulation of the jugular vein and carotid artery and maintained under anesthesia for the remainder of the study. Body temperature was regulated using heating pads, and respiration was visually monitored throughout the experiment. For the first 10 minutes of the insulin infusion, a bolus insulin infusion was given at 32 mU kg^{-1} min^{-1}, twice the constant infusion rate used for the remainder of the experiment. Blood glucose was sampled every 5 minutes, and plasma glucose was clamped at 140 mg/dL. At the end of 90 minutes, the rat was euthanized and plasma samples were processed.

2.3. Determination of muscle ATP synthesis rate and IMCL content

2.3.1. Animal setup

Measurements were carried out in 12- to 14-week–old rats fed either normal chow diet (n = 9) or fat-enriched diet (DIO; D12492i, 60% kcal; Research Diets, NJ; n = 10) from 4 weeks of age. As described in the preliminary study, rats were fasted at 5:00 PM the evening before the experiment; and dual cannulation was performed an hour before imaging. After surgery, the rat was laid prone on a supportive bed for imaging; and the cannula to the jugular vein was connected to 2 pumps, one containing 30% glucose and the other with 800 mU/mL insulin (Eli Lilly) in 0.1% bovine serum albumin in 154 mmol/l NaCl with 20 U/mL heparin, fixed beyond the magnetic field range. The rat was placed at the magnet isocenter and not moved until completion of the experiment. Basal ATP synthesis and the longitudinal (spin-lattice) relaxation time, T_1, for muscle inorganic phosphate (Pi) were measured by ^{31}P magnetic resonance spectroscopy (MRS) as described below. After the baseline measurements, the pumps were activated; and for the first 10 minutes (0-10 minutes), a bolus insulin infusion corresponding to 32 mU kg^{-1} min^{-1} was administered. At 10 minutes, the insulin infusion was reset to 16 mU kg^{-1} min^{-1}, as determined from the preliminary study described above. Blood drawn from the cannulated artery at baseline and then at 5-minute intervals during the infusion was used to monitor blood glucose levels, and the GIR was adjusted to achieve a steady-state blood glucose of 140 mg/dL. While reaching the steady state, IMCL content was measured by localized ^{1}H-MRS. Once the steady state was reached, ATP synthesis rates were again measured by ^{31}P-MRS. After the 90 minutes of euglycemic-hyperinsulinemic clamp, the experiment was stopped and the animal was euthanized.

2.3.2. In vivo MRS

All in vivo magnetic resonance (MR) measurements were performed on a Bruker Avance 7.0 T/30-cm wide-bore instrument (Bruker Medical, Billerica, MA) equipped with a 20-cm internal diameter actively shielded gradient insert. To collect signal from the lower leg of the rat, both ^{1}H and ^{31}P-MRS were performed using a dual-frequency ^{1}H/^{31}P 2.5-cm surface coil working in a transmitter/receiver mode and tuned to 300.31 (^{1}H) and 121.57 (^{31}P) MHz. The MRS data were obtained under 1% to 2% isoflurane anesthesia with constant monitoring of respiration and body temperature (SA Instruments, Stony Brook, NY). On average, total scanning time did not exceed 2.5 hours per animal.

Measurement of the ATP synthesis rate was systematically combined with the quantification of IMCL levels and determination of T_1 for muscle Pi before and during the clamp. After cannulation and pump line connection, the rat leg was carefully secured to the surface coil such that signal was generated mostly from the tibialis anterior (TA) between the knee and ankle. Orthogonal scout images were acquired using a fast imaging with steady-state precession sequence (echo time of 1.82 milliseconds , repetition time of 3.64 milliseconds, slice thickness of 2 mm, field of view of 35 × 35 mm, 8 averages) to confirm proper positioning. The MRS acquisition and subsequent calculations were carried out as described by Laurent et al [4]. A summary of the methods is presented below.

For the ATP synthesis rate determination, 2 spectra were acquired for each saturation transfer experiment, one with and one without (control spectrum) steady-state saturation of the ATPγ peak [6]. Each acquisition consisted of 128 averaged scans, each with a repetition time of 6 seconds, leading to a total acquisition time of 13 minutes per spectrum.