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Acute hyperinsulinemia and reduced plasma free fatty acid levels decrease intramuscular triglyceride synthesis

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ABSTRACT

Objective. To investigate the effect of acute hyperinsulinemia and the resulting decrease in plasma free fatty acid (FFA) concentrations on intramuscular TG synthesis.

Materials/Methods. U-$^{13}C_{16}$-palmitate was infused for 3 h in anesthetized rabbits after overnight food deprivation. Arterial blood and leg muscle were sampled during the tracer infusion. Plasma samples were analyzed for free and TG-bound palmitate enrichments and concentrations. The enrichments and concentrations of palmitoyl-CoA and palmitoyl-carnitine as well as the enrichment of palmitate bound to TG were measured in muscle samples. Fractional synthetic rate (FSR) of intramuscular TG was calculated using the tracer incorporation method. The rabbits were divided into a control group and a hyperinsulinemic euglycemic clamp group. Insulin infusion decreased the rate of appearance of plasma free palmitate (2.00 ± 0.15 vs 0.68 ± 0.20 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; $P < .001$), decreased plasma FFA concentration (327 ± 61 vs 72 ± 25 nmol/mL; $P < .01$), decreased the total concentration of intramuscular fatty acyl-CoA plus fatty acyl-carnitine (12.1 ± 1.6 vs 7.0 ± 0.7 nmol/g; $P < .05$), and decreased intramuscular TG FSR (0.48 ± 0.05 vs 0.21 ± 0.06%/h; $P < .01$) in comparison with the control group. Intramuscular TG FSR was correlated ($P < .01$) with both plasma FFA concentrations and intramuscular fatty acyl-CoA concentrations.

Conclusions. Fatty acid availability is a determinant of intramuscular TG synthesis. Insulin infusion decreases plasma and intramuscular fatty acid availability and thereby decreases TG synthesis.

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1. Introduction

Skeletal muscle accounts for 75%–80% of whole body insulin-mediated glucose uptake and is therefore of central importance in the development of insulin resistance [1–3]. When insulin is ineffective in stimulating skeletal muscle glucose uptake hyperglycemia ensues. Thus, skeletal muscle insulin resistance is closely linked to hyperglycemia and type 2 diabetes [3]. A large

Abbreviations: IM, intramuscular; TG, triglyceride; FFA, free fatty acid(s); CoA, coenzyme A; PalCoA, palmitoyl-CoA; PalCn, palmitoyl-carnitine; PL, phospholipids(s); FSR, fractional synthetic rate(s); GC-MS, gas chromatograph-mass spectrometer.

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body of evidence indicates that muscle insulin resistance is often associated with intramuscular (IM) accumulation of triglyceride (TG) and fatty acid intermediate metabolites [4–7]. IM TG content is inversely related to insulin sensitivity in almost all populations studied except in endurance-trained athletes [8,9]. In order to understand the relationship between intracellular TG accumulation and the metabolic mechanisms underlying the development of insulin resistance it is therefore necessary to measure IM TG kinetics.

The amount of IMTG is determined by the balance between continuous synthesis and degradation. Fatty acid precursors for synthesis of IMTG come in large part from plasma FFA. In this regard, changes in plasma FFA levels are considered to be important in IM lipid dysregulation and insulin resistance [10]. Boden et al [11] reported that lowering of plasma FFA by acute hyperinsulinemia trended to decrease IM TG content, whereas increasing of plasma FFA increased IM TG content in healthy volunteers. Nevertheless, in that experiment the IM TG kinetics were not measured, so it is not known if the changes of IM TG content were due to the response of IM TG synthesis to changes in plasma FFA concentrations.

Isotopic techniques represent the most practical approach to measuring IM lipid kinetics. Several approaches have been published for measuring the fractional synthetic rate (FSR) of IM TG. The common problem of all published methods is measuring the IM precursor for TG synthesis, namely fatty acyl-coenzyme A (CoA). Because of the difficulty in measuring the fatty acyl-CoA, either plasma FFA or IM FFA has been used as surrogates of the precursor without necessary validations [12–16]. In a recent experiment we found that plasma free palmitate overestimated and that IM free palmitate underestimated the true precursor enrichment due to active lipid breakdown *in vivo* and during muscle sampling and processing [17]. IM PalCn enrichment was an acceptable surrogate for IM PalCoA enrichment, because it was less affected by IM lipid breakdown. This finding confirmed that PalCn enrichment can be used as the precursor enrichment for calculating IM TG FSR when direct measurement of IM PalCoA is problematic [18].

The goal of the present experiment was two-fold: to investigate the effect of acute hyperinsulinemia on IM TG synthesis, and to test the usefulness of IM PalCn enrichment as the precursor enrichment for IM TG synthesis. Because acute hyperinsulinemia (without changes in plasma FFA) has been reported to inhibit IM TG synthesis [19] and lowering of plasma FFA tended to decrease IM TG content [20], we anticipated that a combination of hyperinsulinemia and lowering of plasma FFA would inhibit IM TG synthesis.

2. Methods

2.1. Animals

Adult male New Zealand White rabbits (Myrtle's Rabbitry; Thompson Station, TN), weighing ~4.5 kg, were used for this study. The rabbits were housed in individual cages and were given 150 g/day of unpurified diet (Lab Rabbit Chow 5326, Purina Mills; St. Louis, MO) for weight maintenance. This protocol complied with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, incorporated in the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals, and was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

The animals were studied after overnight food deprivation with free access to water. Surgery was performed to insert catheters into the carotid artery and jugular vein under general anesthesia [18]. The arterial line was used for drawing blood and monitoring arterial blood pressure and heart rate; the venous line was used for infusion of anesthetics and saline as well as insulin and glucose. An additional venous line was installed in a marginal ear vein by means of a Teflon-top needle (24 G 3/4 in Introcan® SafetyTM; B. Braum Medical; Bethlehem, PA), which was used exclusively for tracer administration. Tracheotomy was performed for placement of a tracheal tube, which was connected to a hood filled with oxygen-enriched room air.

2.2. Stable isotope tracer infusion

After surgery, we observed blood pressure, heart rate and rectal temperature for 20–30 min to ensure stable physiological conditions before the start of tracer infusion. In the insulin group, regular human insulin was infused at 2.5 $mU \cdot kg^{-1} \cdot min^{-1}$ after a priming dose calculated to raise plasma insulin to 150 μU/mL. Arterial blood was measured every 10 min to adjust the infusion rate of 25% glucose. When the infusion rate of glucose and plasma glucose concentrations were stable, which took 60–90 min, the tracer infusion was started.

U-$^{13}C_{16}$-palmitate (99% enriched; Cambridge Isotope Laboratories), bound to 5% albumin, was infused continuously for 3 h at the dose of ~0.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ after a priming dose of 1.0 μmol/kg. The tracer was infused into the marginal ear vein using a Harvard Syringe pump (Harvard Apparatus; Boston, MA) set at 15 mL/h. Blood samples were taken from the carotid artery catheter at 0 (before the tracer infusion), 5, 30, 60, 90, 120, 150, and 180 min using tubes containing EDTA. After centrifugation plasma was separated and stored at −20 °C for later analysis. Muscle samples were taken from the adductor muscle of both legs at 0, 5, 60, 120, and 180 min. The adductor muscle was dissected to be free from visible facial and adipose tissues before excision with scissors. The muscle samples were washed in ice-cold saline or blotted with soft tissues very briefly, and were frozen in liquid nitrogen immediately using a metal net. After being submerged in liquid nitrogen for 1 min, the frozen muscle samples were transferred to cryogenic tubes and stored at −80 °C.

Mean arterial blood pressure, heart rate and rectal temperature were maintained stable by adjusting the infusion rates of anesthetics and physiological saline, and by using a heating blanket. These vital signs were recorded every 30 min during the 3-h tracer infusion. At the end of the experiment, the rabbits were killed by intravenous injection of 5 mL saturated KCl under general anesthesia.

2.3. Sample analysis

Plasma FFA and TG were processed for measuring palmitate enrichment on a gas chromatograph–mass spectrometer (GC-MS, MSD system, Aligent) [18]. Heptadecanoic acid and

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