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Short-term leptin treatment increases fatty acids uptake and oxidation in muscle of high fat–fed rats☆

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Abstract

The purpose of this study was to measure the effects of short-term (10 days) leptin treatment on insulin sensitivity as it pertains to fatty acid (FA) uptake, oxidation, and muscle triglyceride (mTG) synthesis in animals that have been administered a high-fat (HF) diet for 3 months. Male Wistar rats were randomly assigned to 1 of 4 groups. One group was fed a control diet (CON) and 3 groups were fed a HF diet. The HF and HF-leptin (HF-LEP) groups were fed the HF diet ad libitum and the amount of food eaten by the HF–pair fed (HF-P) group was equal to that of the HF-LEP group. At the end of the dietary period, rats were injected daily either with saline (CON, HF, HF-P) or with leptin (HF-LEP; 10 mg · kg^{-1} · d^{-1}) for 10 days before hindlimb perfusion. The perfusate contained 600 μmol/L palmitate traced with [^{14}C]palmitate, 9 mmol/L glucose, and 100 μU/mL insulin. As dictated by the protocol, energy expenditure was not significantly different ($P > .05$) between HF-LEP and HF-P. Palmitate uptake and oxidation as well as mTG synthesis were greater ($P < .05$) in HF (9.8 ± 0.3, 2.0 ± 0.1, and 1.9 ± 0.2 nmol · min^{-1} · g^{-1}) than in CON (8.0 ± 0.4, 1.4 ± 0.1, and 1.1 ± 0.1 nmol · min^{-1} · g^{-1}) and this was associated with higher levels of mTG in HF. Palmitate uptake and oxidation were higher ($P < .05$) in HF-LEP (10.3 ± 0.6 and 2.0 ± 0.1 nmol · min^{-1} · g^{-1}) than in HF-P (8.3 ± 0.5 and 1.5 ± 0.2 nmol · min^{-1} · g^{-1}, $P < .05$), but mTG synthesis and mTG levels were not changed significantly by leptin treatment ($P > .05$). High-fat feeding decreased glucose uptake by 41% when compared with CON (2.4 ± 0.4 vs 4.1 ± 0.4 μmol · h^{-1} · g^{-1}; $P < .05$) but pair feeding alone (4.7 ± 0.4 μmol · h^{-1} · g^{-1}) or leptin treatment (3.8 ± 0.3 μmol · h^{-1} · g^{-1}) similarly prevented the HF diet–induced decrease in glucose uptake. These data indicate that short-term leptin treatment in HF-fed rats alters muscle FA metabolism by increasing FA uptake and oxidation relative to pair feeding alone. This results in a decrease in the FA esterification-oxidation ratio.

1. Introduction

The impairment of insulin-stimulated glucose uptake by high concentrations of circulating fatty acids (FA) induced by either intralipid infusion or adherence to a high-fat (HF) diet has been well documented [1-4] and suggests that alterations in muscle FA metabolism may be involved in the development of insulin resistance. In line with this notion, muscle triglyceride (mTG) accumulation has been shown to negatively correlate with whole-body insulin-stimulated glucose uptake [5,6]. Although mTG accumulation can occur because of alterations in FA uptake, FA oxidation and/or FA esterification into mTG under both basal and insulin-stimulated conditions, it is not clear which of these metabolic pathways are the most affected by a HF diet. We have demonstrated that 3 weeks of HF feeding (65% fat-derived energy) is accompanied by mTG accumulation, a decrease in glucose uptake, and an increase in FA oxidation in muscle perfused under insulin-stimulated conditions [7]. These results suggest that resistance to the actions of insulin is present in muscle of HF-fed rats not only as it pertains to glucose uptake but also to the antioxidative

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actions of insulin on FA disposal [7]. However, it is not known whether the increase in mTG associated with HF feeding of a longer duration would be associated with further alterations in muscle FA kinetics under insulin-stimulated conditions.

Although it is generally accepted that alterations in FA metabolism are involved in the development of muscle insulin resistance, clinical strategies aimed at preventing the development of these metabolic pathologies are still highly debated [8]. Treatment with leptin is one of the pharmacologic interventions that have been studied because of its ability to improve insulin sensitivity in muscle [9]. Leptin is the 16-kd product of the *ob* gene, which is secreted by adipocytes in proportion to the amount of stored lipid [10]. Early studies have shown that leptin decreases food intake and regulates energy expenditure possibly via inhibition of neuropeptide Y release from the hypothalamus [11]. However, in line with the observation that isoforms of the leptin receptor are expressed in peripheral tissues such as skeletal muscle [12,13], more recent studies have shown that acute leptin administration alters both glucose and FA metabolism in skeletal muscle [14,15]. In isolated mouse soleus muscle incubated without insulin, short-term leptin treatment has been shown to increase FA oxidation and to decrease mTG synthesis [14]. In animals that have been administered a normal diet, short-term (<2 weeks) leptin treatment has also been reported to increase mTG hydrolysis in isolated rat soleus muscle incubated without insulin [16], to decrease FA transport into muscle giant sarcolemmal vesicles [17], and to reduce plasma membrane expression of FAT/CD36 and $FABP_{PM}$ in muscle [17]. These leptin-induced alterations in FA metabolism were associated with a decrease in mTG, suggesting that the insulin sensitizing effects of short-term leptin treatment on muscle glucose uptake [18] may be due in part to alterations in FA metabolism. In HF-fed rats, only the effects of short-term leptin treatment on basal FA metabolism have been studied in skeletal muscle and it was shown that the leptin-induced increase in FA oxidation and decrease in mTG synthesis observed in rats fed with normal rat chow was eliminated in rats fed a HF diet for 4 weeks [19]. These results suggested that with the accumulation of mTG induced by a HF diet, skeletal muscle develops not only insulin resistance but also leptin resistance [19]. Therefore, it is not known whether the insulin sensitizing effects of short-term leptin treatment on muscle glucose uptake observed in HF-fed rats would be associated with alterations in FA metabolism as shown in rats fed with normal rat chow [16,17].

Thus, the aims of this investigation were (1) to evaluate the effects of long-term (3 months) HF feeding on FA metabolism in muscle perfused under insulin-stimulated conditions and (2) to investigate the effects of short-term (10 days) leptin treatment on insulin sensitivity as it pertains to FA uptake, oxidation, and mTG synthesis in animals that have been administered an HF diet for 3 months.

2. Materials and methods

2.1. Animals

Seven-week-old male Wistar rats were randomly assigned to 1 of 4 groups whose initial body mass was not different ($P > .05$) between groups. One group was fed a control (CON) diet and 3 groups were fed a HF diet. The CON group (n = 8) was fed a low fat rat chow containing 4% fat, 24% protein, and 72% carbohydrate (Harlan Teklad, Madison, Wis), and given saline injections twice per day at 8:00 AM and 5:00 PM. The HF group (HF; n = 8) was fed a diet consisting of 65% fat, 22% protein, and 13% carbohydrate (Dyets Inc, Bethlehem, Pa), and was given saline injections twice per day. The HF-leptin (HF-LEP; n = 8) group was fed the HF diet and given leptin (5 mg/kg body weight; Amgen, Inc, Thousand Oaks, Calif) injections twice per day. The HF–pair fed (HF-P; n = 8) group was food restricted to the level of the HF-LEP group and given saline injections twice per day. This 10-day leptin regimen was used because it has been shown to be associated with similar serum leptin levels in CON and HF animals and physiologically elevated leptin levels in HF-LEP animals without a significant body weight discrepancy between the HF and HF-LEP groups [37]. Food intake of all groups was measured daily for the 10-day experimental period. To accomplish pair feeding, the HF-P animals received each day the same amount of HF food as what the HF-LEP animals had consumed the previous day. All animals had ad libitum access to water and were housed on a 12-hour light-dark cycle. Ethical approval for the study was granted from the Institutional Animal Care and Use Committee at the University of Southern California.

2.2. Hindlimb perfusions

On the day of the experiment, animals were anesthetized with an intraperitoneal injection of ketamine/xylazine (80 mg and 12 mg/kg body weight, respectively). Hindlimbs were surgically isolated as previously performed in our laboratory and described in detail [20,21]. Before the perfusion, catheters were inserted and 150 IU heparin was administered to the inferior vena cava. Rats were euthanized immediately before the insertion of the catheters by an intracardial injection of pentobarbital sodium (0.4 mg/g body weight). Immediately after the insertion of the catheters, 25 mL of perfusate was passed through the circulatory system and discarded to reduce possible effects of added heparin on plasma FA availability due to lipoprotein lipase activity. Hindlimbs were perfused for 20 minutes with Krebs-Henseleit solution, 1- to 2-day-old washed bovine erythrocytes (hematocrit 28%) containing 3.5% bovine serum albumin (Cohn