Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance

GREGORY R. STEINBERG,1 MICHELLE L. PAROLIN,2 GEORGE J. F. HEIGENHAUSER,2 AND DAVID J. DYCK1

1Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1; and 2Department of Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

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Steinberg, Gregory R., Michelle L. Parolin, George J. F. Heigenhauser, and David J. Dyck. Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. Am J Physiol Endocrinol Metab 283: E187–E192, 2002.—The adipocyte-derived hormone leptin has been shown to acutely increase fatty acid (FA) oxidation and decrease esterification in resting rodent skeletal muscle. However, the effects of leptin on human skeletal muscle FA metabolism are completely unknown. In these studies, we have utilized an isolated human skeletal muscle preparation combined with the pulse-chase technique to measure FA metabolism with and without leptin in lean and obese human skeletal muscle. Under basal conditions (in the absence of leptin), muscle from the obese demonstrated significantly elevated levels of total FA uptake (+72%, $P = 0.038$) and enhanced rates of FA esterification into triacylglycerol (+102%, $P = 0.042$) compared with lean subjects. In the presence of leptin, lean muscle had elevated rates of endogenous (+103%, $P = 0.01$) and exogenous (+150%, $P = 0.03$) palmitate oxidation. When the ratio of esterification to exogenous oxidation was examined, leptin reduced this ratio (−47%, $P = 0.032$), demonstrating the increased partitioning of FA toward oxidation and away from storage. Contrary to these findings in lean muscle, leptin had no effect on FA metabolism in skeletal muscle of the obese. This study provides the first evidence that leptin increases FA oxidation in skeletal muscle of lean, but not obese humans, thus demonstrating the development of leptin resistance in obese human skeletal muscle.

SKELETAL MUSCLE IS THE MAJOR TISSUE contributing to basal metabolic rate and is also the primary tissue responsible for whole body glucose and fatty acid (FA) metabolism (25). Skeletal muscle from the obese has an increased content of triacylglycerol (TG) (9, 12), which is strongly correlated to reduced insulin sensitivity (12, 17). The mechanisms by which obese individuals increase TG storage in skeletal muscle are currently unknown but may be due to several factors, including 1) increased FA uptake, 2) a repartitioning of incorporated FA toward esterification and away from oxidation, and 3) a reduced rate of TG hydrolysis.

FA metabolism in resting rodent skeletal muscle is regulated by several hormones, including leptin (16, 22), insulin (8), and epinephrine (18). Leptin, a circulating 16-kDa adipocyte-derived protein, regulates food intake and energy expenditure in animal models (10). It has also been proposed that a primary role of leptin may be to prevent the accumulation of lipids in peripheral tissues such as skeletal muscle (23). In agreement with this proposal, we and others have demonstrated both acutely (16, 22) and chronically (21) that, in rodent skeletal muscle, leptin reduces intramuscular TG by increasing FA oxidation and TG hydrolysis while reducing FA esterification. The effects of leptin on human skeletal muscle metabolism, however, are unknown.

Human obesity is characterized by high levels of circulating leptin (14), which suggests the development of central and/or peripheral leptin resistance. We have recently demonstrated that, in rodents, 4 wk of high-fat feeding can induce leptin resistance in skeletal muscle, as demonstrated by the elimination of leptin’s stimulatory effect on FA metabolism (22). Thus we have hypothesized that, during the development of human obesity, skeletal muscle becomes resistant to leptin, contributing to the accumulation of intramuscular TG and the development of insulin resistance. However, direct evidence of leptin resistance in human skeletal muscle does not exist.
Therefore, the principal purpose of this study was to examine the acute effects of leptin on human skeletal muscle FA metabolism. We hypothesized that leptin would stimulate FA oxidation and TG hydrolysis while suppressing FA esterification into TG in lean human skeletal muscle, but that these effects would be blunted in the obese. To monitor FA metabolism, we utilized an isolated rectus abdominis muscle preparation previously described by Dohm et al. (6), combined with the dual-label pulse-chase technique, which has been used to examine FA metabolism in rodent skeletal muscle (7, 18, 22). Unlike a previous study that assessed FA oxidation rates in obese human skeletal muscle homogenates (13), the present study examined FA metabolism in intact human muscle fibers. In this model, all aspects of metabolism are presumably kept intact (i.e., membrane transport, cytoplasmic and mitochondrial metabolism), unlike the homogenate system that essentially examines metabolism downstream of the sarcolemmna (i.e., cytoplasm and mitochondrion). Furthermore, although other studies have examined rates of skeletal muscle FA metabolism in obese humans in vivo (4,11), metabolic complications associated with the obese state and alterations in the hormonal milieu make it difficult to assess the contribution of individual hormonal factors. The isolated muscle preparation used in this study permits precise control of hormone and substrate concentrations, allowing us to study the effects of obesity and leptin in the absence of other metabolic perturbations.

RESEARCH DESIGN AND METHODS

Human Subjects

The participants were eight lean [body mass index (BMI) ≤27 kg/m²; mean, 24.8 ± 0.5 kg/m²] and nine obese (BMI ≥30.0 kg/m²; mean, 33.0 ± 1.5 kg/m²) nondiabetic women (Table 1). Subjects were admitted to McMaster University Ethics Committee. None of the subjects had any diseases or had taken any medications known to alter carbohydrate or lipid metabolism in the 6 mo before surgery. All subjects had maintained a constant body mass during the year preceding surgery. After an overnight fast (12–18 h), general anesthesia was induced with a short-acting barbiturate and maintained by a fentanyl and rocuronium volatile anesthetic mixture. Venous blood samples were then collected in 5-ml heparinized tubes during anesthesia. Muscle strip preparation was completed as previously described (6), with minor modifications. A biopsy of 3 × 2 × 1 cm from the rectus abdominis muscle was excised perpendicular to the direction of the muscle fibers, clipped at resting length, and placed in oxygenated, ice-cold Krebs-Henseleit buffer for transport to the laboratory (~5 min). Four muscle strips weighing ~25 mg were separated from the muscle sample and clipped at resting length.

Muscle Viability

In preliminary experiments, ATP, phosphocreatine (PCr), and total creatine (Cr) were measured spectrophotometrically (2) to ensure muscle viability. Muscle strips were rapidly frozen in liquid N₂ after excision (control) or after 90 min of incubation under the same conditions in which pulse-chase experiments were conducted.

Pulse-Chase Studies

Muscle strips were placed in a 20-ml glass scintillation vial containing 3 ml of warmed (30°C), pregassed (95% O₂-5% CO₂, pH 7.4) modified Krebs-Henseleit buffer containing 4% FA-free BSA (ICN Biomedicals), 2 mM pyruvate, and 0.5 mM palmitate. This was the base buffer used in all experiments. Throughout the experiment, vials were gently shaken in a water bath. Pulse-chase procedures and lipid extractions were completed as previously described (22). After a preincubation period of 20 min, muscles were transferred to a pulse buffer containing 0.5 μCi/ml of [9,10-³H]palmitate (Amersham, Oakville, ON, Canada) for 40 min to prelabel endogenous lipid pools [TG, diacylglycerol (DG), phospholipid (PL)]. Subsequently, muscles were washed for 30 min to remove all nonincorporated radiolabeled palmitate, and one strip was removed for lipid extraction. The remaining muscle strips were transferred to the experimental or chase phase containing 0.5 μCi/ml of [1-14C]palmitate (Amersham) under either 1) control or 2) leptin conditions (10,000 ng/ml recombinant human leptin; Amgen, Thousand Oaks, CA). This concentration has previously been demonstrated to elicit a maximal response in rodent skeletal muscle FA metabolism (16). During the 60-min chase phase, exogenous palmitate oxidation and esterification were monitored by the production of 14CO₂ and incorporation of [1-14C]palmitate into endogenous lipids, respectively. Intramuscular lipid hydrolysis was simultaneously monitored by measuring the decrease in lipid [³H]palmitate content.

Extraction of Muscle Lipids

Muscles were placed in 13-ml plastic centrifuge tubes containing 5.0 ml of ice-cold 1:1 chloroform-methanol (vol/vol) and homogenized with a polytron (Brinkman Instruments, Mississauga, ON, Canada). After homogenization, connective tissue was removed, weighed, and subtracted from the total wet weight. Samples were then centrifuged at 2,000 g (4°C) for 10 min. The supernatant was removed with a glass Pasteur pipette and transferred to a clean centrifuge tube. Distilled water (2.0 ml) was added, and samples were shaken for 10 min and centrifuged as before to separate the aqueous and lipophilic phases. A 0.5-ml aliquot of the aqueous phase was quantified by liquid scintillation counting to determine the amount of ¹⁴C-labeled oxidative intermediates resulting from isotopic fixation. This acid-soluble metabolite contribution to total lipid oxidation was determined in every sample and was approximately equivalent to the contribution from

| Table 1. Clinical characteristics and fasting plasma values of lean and obese women |
|-----------------|-----------------|-----------------|
| Lean            | Obese           | P Value         |
| Age, yr         | 48.7 ± 4.0      | 55.1 ± 6.4      | NS              |
| Weight, kg      | 66.4 ± 16       | 89.6 ± 3.2      | <0.001          |
| Body mass index, kg/m² | 24.8 ± 0.5     | 33.0 ± 1.5      | <0.001          |
| Glucose, mM     | 6.1 ± 0.3       | 6.0 ± 0.3       | NS              |
| Free fatty acids, mM | 1.00 ± 0.16    | 1.02 ± 0.23     | NS              |
| Glycerol, mM    | 0.09 ± 0.02     | 0.15 ± 0.01     | 0.03            |
| β-Hydroxybutyrate, mM | 0.53 ± 0.12  | 0.38 ± 0.16     | NS              |
| Insulin, μU/ml  | 8.32 ± 1.12     | 9.38 ± 3.69     | NS              |
| Leptin, ng/ml   | 8.20 ± 1.77     | 19.74 ± 1.6     | 0.002           |

Values are means ± SE; n = 6–9. NS, not significant.
Measurement of Endogenous and Exogenous Oxidation

Tritiated H2O produced from the endogenous oxidation of [9,10-3H]palmitate was separated from the labeled substrate by transferring 1.0 ml of the chase incubation medium to a plastic centrifuge tube containing 5.0 ml of 2:1 chloroform-methanol (vol/vol). Samples were then shaken for 10 min before addition of 2.0 ml of 2 M KCl-HCl, shaken again for 10 min, and then centrifuged at 2,000 g at 4°C for 15 min. A 0.5-ml aliquot was removed from the aqueous phase and quantified by liquid scintillation counting.

Gaseous 14CO2 produced from the exogenous oxidation of [1-14C]palmitate during the incubation was measured by transferring 1.0 ml of the chase incubation medium to a 20-ml glass scintillation vial containing 1.0 ml of 1 M H2SO4 and a 0.5-ml Fisher microcentrifuge tube containing 300 μl benzethonium hydroxide. Liberated 14CO2 was trapped in the benzethonium hydroxide for 60 min, and the microcentrifuge tube containing trapped 14CO2 was placed in a scintillation vial and counted.

Calculations and Statistics

The absolute quantity of palmitate esterified and oxidized was based on the ratio of labeled palmitate to total palmitate in the incubation medium. Hydrolysis of intramuscular lipids at rest was calculated from the loss of preloaded [3H]palmitate (nmol palmitate/g wet wt) from each pool during the chase (i.e., the loss of [3H]palmitate content from the pulse to the end of the chase). Total palmitate uptake was calculated as the sum of [14C]palmitate deposition into all lipid pools and total lipid oxidation. All data are reported as means ± SE. Results were analyzed using analysis of variance (ANOVA) procedures, and Tukey’s post hoc test was used to test significant differences revealed by the ANOVA. Significance was accepted at P ≤ 0.05.

RESULTS

Subjects

Subject characteristics are shown in Table 1. Obese individuals had significantly greater body mass (+34%, P < 0.001) and BMI (+35%, P < 0.001) compared with lean subjects. Fasting levels of plasma glucose, fatty acids, β-hydroxybutyrate, and insulin were not significantly different between lean and obese women (P > 0.05). Leptin levels were significantly elevated (+140%, P = 0.002) in obese individuals and were positively correlated with BMI (r = 0.73, P = 0.016), as previously demonstrated (5).

Muscle Strip Preparation Viability

Muscle viability was preserved during incubations on the basis of maintenance of ATP (pre, 19.2 ± 1.3; post, 17.0 ± 1.3 μmol/g dry wt), PCr (pre, 51.8 ± 1.3; post, 47.4 ± 2.6 μmol/g dry wt), and total Cr (pre, 78.1 ± 1.1; post, 70.4 ± 3.9 μmol/g dry wt) contents.

Skeletal Muscle FA Metabolism

Lean vs. obese. In the absence of leptin, exogenous and endogenous palmitate oxidation was not different between lean and obese subjects (Fig. 1, A and B). However, obese muscle had significantly higher levels of total FA uptake (+72%, P = 0.038; Fig. 1C), primarily due to increased rates of FA esterification into TG (+102%, P = 0.042; Fig. 1D). FA esterification into PL was significantly elevated (+96%, P = 0.031) in the obese, whereas esterification into DG was not different (data not shown). There was no change in the hydrolysis of TG, DG, or PL between lean and obese groups (data not shown). Total FA uptake (r = 0.54, P = 0.027) and esterification to TG (r = 0.58, P = 0.014) were positively correlated with BMI (Fig. 2). The ratio of palmitate esterification (PL, DG, TG) to oxidation was significantly elevated in obese muscle (+49%, P = 0.05), indicating a partitioning of incorporated FA toward storage (Fig. 3).

Metabolic responses to leptin in lean human skeletal muscle. Exogenous and endogenous palmitate oxidation (Fig. 1, A and B) was significantly elevated (+150 and +103%, respectively, P < 0.03) in the presence of leptin. Leptin had no effect on FA uptake (Fig. 1C), TG esterification (Fig. 1D), or the hydrolysis of TG and DG pools (data not shown). Leptin treatment resulted in a significant reduction in the FA esterification-to-oxidation ratio (−50%, P = 0.016; Fig. 3).

Metabolic responses to leptin in obese human skeletal muscle. FA oxidation was not increased by leptin in obese skeletal muscle strips (Fig. 1, A and B). Leptin was also without effect on total FA uptake (Fig. 1C), TG esterification (Fig. 1D), or the hydrolysis of TG and DG pools (data not shown) or the esterification-to-oxidation ratio (Fig. 3).

DISCUSSION

In this study we used the pulse-chase technique in isolated human skeletal muscle from lean and obese women to examine the effect of obesity and leptin on FA metabolism. Our results demonstrate that 1) in obese human skeletal muscle, there is a significantly greater rate of FA uptake and esterification compared with skeletal muscle of lean individuals, and 2) leptin stimulates FA oxidation in skeletal muscle from lean, but not obese, subjects providing the first direct evidence of peripheral leptin resistance in obese humans. Thus an increased rate of FA uptake and esterification,
coupled with an inability for leptin to stimulate FA oxidation, may contribute to the increased intramuscular TG content observed in obesity. Although one of the benefits of the isolated muscle preparation is the ability to precisely control hormone and substrate concentrations and thus establish a direct effect of leptin, it should also be noted that the nonphysiological conditions imposed in such a preparation (i.e., high leptin, absence of insulin and other hormonal factors) make it difficult to extrapolate our findings to the in vivo condition. Thus, although our findings may have a significant impact regarding our understanding of the development of insulin resistance in human skeletal muscle, further studies will be necessary to establish the causative role of leptin resistance in vivo.

Differences in Basal Rates of FA Metabolism Between Lean and Obese Subjects

In this study, there was a significantly greater rate of FA uptake and TG esterification in skeletal muscle of the obese. However, obesity did not result in altered basal rates of endogenous or exogenous FA oxidation. Taken together, these results cause an elevated esterification-to-oxidation ratio in obese human skeletal muscle, indicating a greater rate of FA storage, and they are in agreement with previous findings demonstrating elevated levels of intramuscular TG with obesity (9, 12). Previous studies have demonstrated increased content of skeletal muscle FA transporters in obese rats (FAT/CD36 and FABPpm) (15) and humans (FABPpm) (20), suggesting that the enhanced rate of FA esterification is due at least in part to increased FA transport.

Our finding of unaltered rates of FA oxidation differs from previous reports of reduced FA oxidation in the obese state (11, 13). We have previously demonstrated that insulin reduces FA oxidation by 38% in resting rodent skeletal muscle (8), and in humans, insulin reduces whole body FA oxidation by 63% (1). Thus reduced rates of FA oxidation previously reported in human obesity (11) may be due in part to insulin’s suppression of FA oxidation. Reduced rates of FA oxidation have also been reported in muscle homogenates from obese humans in the absence of insulin (13). However, it is difficult to compare the findings of Kim et al. (13) with our own because of the disruption of the
skeletal muscle TG by chronic leptin treatment is associated with increased insulin sensitivity (24). We have previously shown that leptin reduces skeletal muscle TG by reducing TG esterification and increasing FA oxidation and TG hydrolysis in oxidative rodent skeletal muscle (22). In this study, we demonstrated for the first time a direct effect of leptin on human skeletal muscle. Leptin significantly enhanced both endogenous and exogenous rates of FA oxidation, but it failed to increase TG hydrolysis or suppress FA esterification, suggesting a possible species difference. Importantly, leptin did not stimulate FA oxidation in skeletal muscle of obese women, demonstrating the development of peripheral leptin resistance.

Human obesity is characterized by elevated serum leptin levels (5), leading to the hypothesis that obese persons are resistant to leptin. In rodents, high-fat diets increase serum leptin levels and reduce leptin sensitivity in skeletal muscle (22). In rodents, recombinant leptin administration is associated with dose-dependent reductions in food intake and body weight (9); however, the effect of recombinant leptin administration in obese humans has been less successful (10, 24). Our findings provide direct evidence of leptin resistance in human skeletal muscle. Although the cause of leptin resistance in obese humans remains unknown, future studies may examine the expression of SOCS-3, a member of the suppressors of cytokine signaling family, which has been demonstrated to potentially inhibit leptin signaling in rodents (3).

In summary, the present study has demonstrated that obese human skeletal muscle has significantly greater rates of FA uptake than muscle from lean subjects and that leptin fails to stimulate FA oxidation in the obese. This reduced capacity for leptin to stimulate FA oxidation, coupled with an increased potential for FA uptake, may contribute to the accumulation of intramuscular TG commonly observed in obesity. Although the mechanism by which skeletal muscle becomes resistant to leptin is unknown, our data indicate that resistance occurs at an early stage in the development of obesity and may precede impairments in FA oxidation.
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