Fatty acid oxidation and triacylglycerol hydrolysis are enhanced after chronic leptin treatment in rats

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Steinberg, Gregory R., Arend Bonen, and David J. Dyck. Fatty acid oxidation and triacylglycerol hydrolysis are enhanced after chronic leptin treatment in rats. Am J Physiol Endocrinol Metab 282: E593–E600, 2002. First published November 13; 10.1152/ajpendo.00303.2001.—Leptin acutely increases fatty acid (FA) oxidation and triacylglycerol (TG) hydrolysis and decreases TG esterification in oxidative rodent muscle. However, the effects of chronic leptin administration on FA metabolism in skeletal muscle have not been examined. We hypothesized that chronic leptin treatment would enhance TG hydrolysis as well as the capacity to oxidize FA in soleus (SOL) muscle. Female Sprague-Dawley rats were infused for 2 wk with leptin (LEPT; 0.5 mg·kg⁻¹·day⁻¹) by use of subcutaneously implanted miniosmotic pumps. Control (AD-S) and pair-fed (PF-S) animals received saline-filled implants. Subsequently, FA metabolism was monitored for 45 min in isolated, resting, and contracting (20 tetani/min) SOL muscles by means of pulse-chase procedures. Food intake (-33 ± 2%, P < 0.01) and body mass (-12.5 ± 4%, P = 0.01) were reduced in both LEPT and PF-S animals. Leptin levels were elevated (+418 ± 7%, P < 0.001) in treated animals but reduced in PF-S animals (-73 ± 8%, P < 0.05) relative to controls. At rest, TG hydrolysis was increased in leptin-treated rats (1.8 ± 2.2, AD-S vs. 23.5 ± 8.1 nmol/g wet wt, LEPT; P < 0.001). In contracting SOL muscles, TG hydrolysis (1.5 ± 0.6, AD-S vs. 3.6 ± 1.0 μmol/g wet wt, LEPT; P = 0.02) and palmitate oxidation (18.3 ± 6.7, AD-S vs. 45.7 ± 9.9 nmol/g wet wt, LEPT; P < 0.05) were both significantly increased by leptin treatment. Chronic leptin treatment had no effect on TG esterification either at rest or during contraction. Markers of overall (citrate synthase) and FA (hydroxyacyl-CoA dehydrogenase) oxidative capacity were unchanged with leptin treatment. Protein expression of hormone-sensitive lipase (HSL) was also unaltered following leptin treatment. Thus leptin-induced increases in lipolysis are likely due to HSL activation (i.e., phosphorylation). Increased FA oxidation secondary to chronic leptin treatment is not due to an enhanced oxidative capacity and may be a result of enhanced flux into the mitochondrion (i.e., carnitine palmitoyltransferase I regulation) or electron transport uncoupling (i.e., uncoupling-protein-3 expression).

In this study, we utilized the dual-label pulse-chase technique to investigate the effect of chronic (2 wk), moderate hyperleptinemia on FA metabolism in resting and contracting rat soleus (SOL) muscle. It should be stressed that, unlike previous studies that have examined FA metabolism in isolated muscles in the presence of pharmacological levels of leptin (23, 24, 31), the present experiments did not include leptin in the incubation medium. Therefore, we have assumed that any changes in FA metabolism would be the result of previous chronic exposure to leptin; however, it must be acknowledged that secondary effects, such as a decrease in circulating insulin levels as a result of hyperleptinemia, may also be a factor. We hypothesized that, in resting and contracting oxidative skeletal muscle, 2 wk of leptin administration would 1) increase TG hydrolysis and FA oxidation and 2) decrease the rate of FA esterification into TG. Furthermore, we hypothesized that leptin would alter FA metabolism pronounced effects on insulin sensitivity independent of calorie restriction (19). This suggested that leptin may have significant metabolic effects on peripheral tissues such as skeletal muscle. This has been confirmed in several recent rodent studies, in which leptin has been shown to acutely (<1 h) increase fatty acid (FA) oxidation (24, 25, 33) and triacylglycerol (TG) hydrolysis (33), while decreasing FA esterification into TG in resting skeletal muscle (24, 25, 33). However, the chronic effects of leptin treatment on skeletal muscle FA metabolism have not been examined to date.

Chronic leptin administration results in the depletion of muscle and pancreatic TG stores (8, 39), but whether this is due to increased rates of TG hydrolysis or lowered rates of FA esterification has not been addressed. Similarly, whether FA oxidation is altered in skeletal muscle by chronic hyperleptinemia has also not been examined. Reduced intramuscular TG stores have been associated with improved insulin sensitivity in both rats (8, 34) and humans (26, 28). Therefore, it is important to understand how chronic leptin treatment alters concentrations of intramuscular TG, as this may have important implications for the correction of insulin resistance.

In initial studies with ob/ob mice demonstrated that leptin caused a rapid reduction in food intake as well as

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through 3) enhanced expression of hormone-sensitive lipase (HSL) and 4) increased oxidative capacity, as indicated by citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activities.

METHODS

Animals

Rats were randomly assigned to one of the following groups: ad libitum fed, saline treated (AD-S), pair fed, saline treated (PF-S), or leptin treated (LEPT). Animals were anesthetized with halothane, and a small incision was made through the skin on the upper region of the back between the scapulae. Miniosmotic pumps (2ML2, Durect, Cupertino, CA) were filled with either sterile phosphate-buffered saline (AD-S, PF-S) or murine leptin (donated by Amgen, Thousand Oaks, CA) and inserted through the incision. The incision was closed with a single autoclip. A leptin dosage of 0.5 mg·kg⁻¹·day⁻¹ was used, because this had previously been demonstrated to induce moderate hyperleptinemia (3, 21). Animals were then assigned to individual cages and maintained on a reverse 12:12-h light-dark cycle. Water was freely accessible to all groups. Food intake was ad libitum for both the AD-S and LEPT animals, whereas PF-S animals were fed the same amount of chow as the LEPT animals. This was determined by measuring the amount of food remaining and the food spillage. Body mass was monitored over the 2-wk treatment period. The Committee on Animal Care at the Universities of Waterloo and Guelph approved all procedures. Two sets of implantation experiments were run in parallel: 1) one set (n = 6–8 per group) for the determination of blood insulin, leptin, and PFA, as well as muscle TG, CS, β-HAD, and HSL, and 2) the other set for pulse-chase experiments monitoring SOL FA metabolism (n = 8 per group).

Blood and Tissue Sampling

Blood was collected at the completion of treatments (2 wk) via cardiac puncture, after the excision of SOL muscle as described below. All samples were taken while rats were in the fed state, between 0900 and 1100, to eliminate variability caused by diurnal rhythm. Blood was transferred to microcentrifuge tubes, where it was allowed to clot before being centrifuged (12,000 g) and the serum collected. Serum leptin and insulin levels were assayed in duplicate using RIA kits specific for rat leptin and insulin (Linco, St. Charles, MO). FA were assayed using a Wako NEFA kit (Wako Chemical, Richmond, VA) and measured on the spectrophotometer at 550 nm. Serum glucose levels were determined fluorometrically (4). SOL muscle TG content was determined on freeze-dried samples, which were dissected free of all visible connective tissue and blood, as previously outlined (18).

Enzyme Measurements

Enzyme activity of CS and β-HAD were measured spectrophotometrically in SOL muscle obtained from a separate set of experiments. SOL muscle was excised and frozen in tongs precooled in liquid N₂ and stored at −80°C until analyzed. The frozen muscle was homogenized in a 20 mM phosphate-glycerol buffer (pH 7.4) and analyzed for maximal activity at 25°C, as previously described (10). Briefly, CS activity was determined by initiating the reaction with oxaloacetate and measuring the production of NAD⁺. β-HAD activity was determined by initiating the reaction with S-acetylacte-CoA and measuring the production of NAD⁺.

Pulse-Chase Studies

Preincubation (equilibration). After 2 wk of treatment (leptin or saline), rats were anesthetised with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body wt), and the SOL muscle was carefully dissected into longitudinal strips from tendon to tendon with a 27-gauge needle. Two strips were utilized from each SOL muscle. Tendons were sutured, and the strip was removed and mounted in a 7-ml incubation reservoir. The sutures were secured to brass hooks to maintain resting tension in the muscle. Seven milliliters of warmed (30°C), pregressed (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 were immediately added to the incubation reservoir; this was the base buffer used in all experiments. The temperature was maintained at 30°C, and the incubation medium was continuously gassed.

Preexperimental labeling of the intramuscular lipid pools. The pulse-chase procedures used have been described previously (16). Briefly, the preincubation buffer was drained, and a pulse buffer consisting of the base buffer plus 2 µCi of [9,10-3H]palmitate (Amersham Life Sciences, Oakville, ON, Canada) was added to the reservoir. Muscles were pulsed with [9,10-3H]palmitate for 40 min to prelabel all endogenous lipid pools [TG, diacylglycerol (DG), phospholipid (PL)]. The pulse buffer was drained, and muscles were washed for 30 min with incubation medium containing no radiolabeled palmitate. At the end of the pulse and wash, one SOL strip from each pair was removed, blotted, weighed, and extracted for endogenous lipids, as described below, to determine the incorporation of [9,10-3H]palmitate.

Experimental phase (chase). The remaining muscles were incubated for an additional 45 min with 0.5 µCi/ml [1-14C]palmitate (Amersham Life Science) at rest or while stimulated to contract at 20 tetani/min (150-ms train, 60 Hz, 20–40 V). During the 45-min chase phase, endogenous palmitate oxidation and esterification were monitored by the production of 14CO₂ and incorporation of [1-14C]palmitate into endogenous lipids. Intramuscular lipid hydrolysis was simultaneously monitored by measuring the decrease in lipid [14C]palmitate content.

Extraction of Muscle Lipids

Muscles were placed in 13-ml plastic centrifuge tubes containing 5.0 ml of ice-cold chloroform-methanol (1:1 vol/vol) and homogenized using 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. One milliliter of the aqueous phase was quantified by liquid scintillation counting to determine the amount of [14C]-labeled oxidative intermediates resulting from isotopic fixation. This represented a twofold correction factor for exogenous [14C]palmitate oxidation, as previously described (16, 32).

The chloroform phase, which contains the total lipids extracted from muscle, was gently evaporated under a stream of N2 and redissolved in 100 µl of 2:1 chloroform-methanol. A small amount of phosphatidylcholine, dipalmitin, and tripalmitin (Sigma Chemical, St. Louis, MO) was added to the 2:1 chloroform-methanol to facilitate the identification of lipid bands on the silica gel plates. Fifty microliters of each sample were spotted onto an oven-dried silica gel plate (Fisher Scientific Canada, Mississauga, ON, Canada). Silica gel plates were placed in a sealed tank containing solvent (heptane-isopropyl ether-acetic acid, 60:40:3) for 40 min. Plates were then permitted to dry and were sprayed with dichlorofluorescein dye (0.02% wt/vol in ethanol) and visualized under long-wave ultraviolet light. The individual lipid bands were marked on the plate with a scalpel and scraped into vials for liquid scintillation counting.

Measurement of Exogenous Oxidation

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 1 M benzenethionium hydroxide. Liberated 14CO2 was trapped in the benzethionium hydroxide over 60 min, and the microcentrifuge tube containing trapped 14CO2 was placed in a scintillation vial and counted.

Calculations and Statistics

The quantity of palmitate esterified and oxidized was calculated from the specific activity of labeled palmitate in the incubation medium (i.e., radiolabeled palmitate in dpm/total palmitate in nmol). Hydrolysis of intramuscular lipids at rest was calculated from the loss of preloaded [3H]palmitate (in nmol) and oxidized was calculated from the loss of each nanomole of incorporated [3H]palmitate representing a twofold correction factor for exogenous [14C]palmitate oxidation, as previously described (16, 32).

RESULTS

Effects of Chronic Leptin Treatment

Body composition and food intake. Food intakes were significantly reduced in LEPT animals compared with the AD-S group (−33%, P < 0.01; Table 1). Food intake in PF-S animals was matched with that in LEPT animals. Food intake was constant over the 2-wk treatment period in all groups. Body mass was reduced in both LEPT and PF-S animals (−12.5%, P = 0.01) after 2 wk of treatment (Table 1).

Serum. Relative to Ad-S, serum leptin was elevated in LEPT animals (+418%, P < 0.001), while being reduced (−73%, P < 0.05) in the PF-S group (Fig. 1A). Serum levels of insulin and FA (Table 2) were significantly reduced (−85%, P < 0.001 and −49%, P = 0.05, respectively) by chronic leptin treatment compared with AD-S. Insulin and FA levels of PF-S animals were unchanged. Glucose levels remained unchanged in LEPT and PF-S animals (Table 2).

Muscle. Intramuscular TG in SOL was significantly reduced in LEPT animals relative to AD-S (−41%, P = 0.03) and PF-S (−33%, P = 0.05) groups (Fig. 1B). Intramuscular TG from PF-S animals was not significantly different from that in AD-S animals. There was a significant inverse correlation between serum leptin and intramuscular TG content (r = 0.71, P < 0.01; Fig. 1C).

Enzyme activity. Despite large increases in lipid oxidation (see below), chronic leptin treatment had no effect on the maximal activity of CS or HSL (Fig. 2; Table 3). HSL expression was also unaltered after chronic leptin treatment (Fig. 2; Table 3).

Metabolic Responses to Chronic Leptin Treatment

Exogenous palmitate oxidation and esterification in SOL. Chronic leptin treatment had no effect on exogenous palmitate oxidation (Fig. 3) in resting SOL muscle. However, during contraction, palmitate oxidation was significantly greater in LEPT animals relative to both AD-S and PF-S animals (Fig. 3). Prior exposure to leptin reduced TG esterification at rest (relative to PF-S animals) but not in contracting muscle (Table 4). Esterification of palmitate into the PL and DG pools was also unaffected by leptin (Table 4). Total FA uptake (PL + DG + TG + oxidation; Fig. 4) tended to be reduced by leptin at rest (−25%) and during contraction (−7%) relative to PF-S animals, but these differ-

Table 1. Body mass and food intake before and after 14 days of the treatment period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 0</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AD-S</td>
<td>PF-S</td>
</tr>
<tr>
<td>Body mass, g</td>
<td>248 ± 3</td>
<td>245 ± 6</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>18.3 ± 0.5</td>
<td>19.0 ± 0.3</td>
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Values are means ± SE; n = 12 animals per treatment. AD-S, ad libitum fed, saline treated; PF-S, pairfed, saline treated; Leptin, leptin treated. *Significantly different from AD-S.
ences were not significant. Conversely, there was a trend toward increased total FA uptake in both resting (+18.5%, *P* = 0.220) and contracting (+29%, *P* = 0.064) muscle from PF-S rats, compared with the ad libitum-fed group (Fig. 4).

**Intramuscular lipid hydrolysis**. Chronic leptin treatment significantly enhanced the rate of TG hydrolysis in both resting (*P* < 0.001) and contracting (*P* < 0.001) SOL muscle (Fig. 5). Hydrolysis of the PL and DG pools was negligible in PL and DG pools (i.e., <5 mmol·g^-1·45 min^-1) and was generally unaffected by leptin treatment (data not shown).

**DISCUSSION**

Previous studies (8, 39) have demonstrated that chronic leptin treatment reduces TG levels in oxidative rodent skeletal muscle and pancreatic islets, but the mechanism(s) by which this occurs is unknown. In this study, we utilized an isolated skeletal muscle preparation to assess changes in FA metabolism as a result of chronic, in vivo leptin treatment at a physiological concentration. This study differs from previous work by us (33) and others (24, 25) that have demonstrated an acute stimulatory effect on FA metabolism in the presence of pharmacological levels of leptin. It is important to stress that, in the present study, FA metabolism was actually monitored in the absence of leptin in the incubation medium after the 2-wk period of chronic hyperleptinemia. Thus the observed alterations in FA metabolism are a consequence of prior leptin treatment and not due to an acute effect. Because chronic exposure to leptin results in a reduction of plasma insulin, we must acknowledge the possibility that our observed changes in muscle FA metabolism may be a secondary effect due to reduced insulin as opposed to a primary effect due to increased leptin. However, it is our belief that the increase in leptin is physiologically more important, and we have discussed this aspect in greater detail below.

Chronic leptin treatment was found to 1) reduce intramuscular TG stores in vivo, 2) increase TG hydrolysis at rest and during contraction, and 3) increase palmitate oxidation during contraction. CS and β-HAD activity, as well as HSL expression, were unaltered after 2 wk of leptin treatment, suggesting that increased rates of FA oxidation may be due to increased

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AD-S</th>
<th>PF-S</th>
<th>Leptin</th>
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<tr>
<td>Insulin, ng/ml</td>
<td>1.36 ± 0.033</td>
<td>1.07 ± 0.30</td>
<td>0.18 ± 0.04†</td>
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<tr>
<td>FA, mmol</td>
<td>0.34 ± 0.04</td>
<td>0.38 ± 0.11</td>
<td>0.17 ± 0.02†</td>
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<tr>
<td>Glucose, mmol</td>
<td>5.20 ± 0.21</td>
<td>5.14 ± 0.41</td>
<td>5.04 ± 0.48</td>
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</tbody>
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Values are means ± SE; *n* = 12 animals per treatment. FA, fatty acid. †Significantly different from AD-S; ‡significantly different from PF-S.

**Table 3. Enzyme activity of CS and β-HAD in SOL measured at 24°C, and HSL protein concentration in soleus muscle (SOL) determined by Western blotting**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>AD-S</th>
<th>PF-S</th>
<th>Leptin</th>
</tr>
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<tbody>
<tr>
<td>CS</td>
<td>23.3 ± 0.4</td>
<td>23.5 ± 0.5</td>
<td>23.8 ± 0.7</td>
</tr>
<tr>
<td>β-HAD</td>
<td>3.1 ± 0.4</td>
<td>3.1 ± 0.3</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>HSL</td>
<td>100 ± 11.0</td>
<td>88.2 ± 11.7</td>
<td>93.3 ± 9.7</td>
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</table>

Values of citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) are means ± SE and are reported in mmol/g wet muscle; *n* = 12 animals per treatment. Hormone-sensitive lipase (HSL) protein expression is expressed as percent AD-S HSL protein density; *n* = 8.

Fig. 1. Serum leptin concentrations (A), soleus (SOL) intramuscular triacylglycerol (TG) (B); and linear regression of serum leptin vs. intramuscular TG (C) after 2-wk treatment period. *Significantly different from ad libitum-fed, saline-treated rats (AD-S); †significantly different from pair-fed, saline-treated rats (PF-S).
carnitine palmitoyltransferase I (CPT-I) activity, whereas increased TG hydrolysis is likely the result of covalent activation of HSL (i.e., phosphorylation). This is the first study to directly demonstrate that chronic leptin treatment enhances FA oxidation and TG hydrolysis in oxidative skeletal muscle, which may explain leptin’s ability to reduce intramuscular TG.

Effects of Chronic Leptin Treatment on Serum and Muscle Parameters

Several studies have examined the effects of chronically elevated leptin levels by use of either miniosmotic pumps (2, 3, 11, 37), as in the present study, or via injection of recombinant adenovirus-containing leptin cDNA (8). In this study, we obtained moderate levels of hyperleptinemia (~5-fold increase), a level that is similar to that obtained with high-fat feeding in rodents (33). Therefore, unlike numerous other studies that have used pharmacological levels of leptin (11, 37, 39), the levels used in this study are within physiological limits.

Serum FA. One of the characteristics of obesity is the presence of high circulating FA. We (present study) and others (11) have demonstrated that chronic leptin treatment (12–14 days) results in a significant reduction in circulating FA. This may be due to one of two possible mechanisms: 1) selective depletion of the labile visceral adipose stores (3) or 2) retention of the FA within the adipocyte for oxidation as a result of increased activity of CPT-I, acyl-CoA oxidase, and uncoupling protein (UCP)-1 expression in this tissue (12, 29, 30).

Serum insulin. Acutely, leptin reduces insulin secretion in isolated pancreatic islet cells (9, 40). In this study, chronic leptin treatment reduced serum insulin. However, circulating glucose levels did not become elevated as a consequence, which suggests that insulin sensitivity may have improved. This has been demonstrated in several studies (3, 8, 11, 37–39) and may be due to the leptin-induced decrease in intramuscular TG (39). The relationship between elevated intramuscular TG and impaired insulin sensitivity has been demonstrated in rodents (8, 34) and humans (26, 28), although the underlying mechanisms are unknown.

Effect of Chronic Leptin Treatment on Lipid Metabolism in Skeletal Muscle

The first site of regulation of FA metabolism in muscle is transport across the sarcolemma, mediated by fatty acid translocase (FAT/CD36) and plasma membrane fatty acid-binding protein (FABPpm) (7). FAT/CD36 expression can be altered by perturbations in metabolism such as chronic muscle stimulation (5) and obesity (D. J. Dyck and A. Bonen, unpublished findings). We have recently demonstrated that the expression of FAT/CD36 can also be regulated acutely through translocation of the transporter from intracellular stores to the plasma membrane during contraction (6). In this study, there was a trend toward chronic leptin treatment reducing total FA uptake (esterification + exogenous oxidation) in resting skeletal muscle. This is in agreement with our recent observations (D. J. Dyck and A. Bonen, unpublished findings) that chronic leptin administration reduces both FAT/CD36 and FABPpm content in the sarcolemma (33a). However, this effect is nearly eliminated during contraction, supporting our observation that FAT/CD36 is translocated from intracellular compartments to the plasma membrane by contraction, thus increasing FA uptake (6).

Contrary to the finding observed in LEPT animals, PF-S rats that were calorically restricted by 33% over the 2-wk treatment period displayed a trend toward increased FA uptake under both resting (+18.5%, P = 0.22) and contracting (+29%, P = 0.064) conditions. This finding is in agreement with work by Turcotte et al. (35), demonstrating increased FABPpm content in red skeletal muscle after 48 h of fasting. It is tempting to speculate that the lower serum leptin level during caloric restriction is responsible for an increase in the expression of the FA transporters, resulting in an elevated FA uptake. However, there are clearly numerous hormonal changes that occur during fasting, making it impossible to definitively comment on the isolated effects of reduced leptin levels.

In this study, leptin increased exogenous FA oxidation by >200% in contracting SOL muscle. However, in resting isolated SOL muscle, there was no observable chronic effect of leptin on FA oxidation. At rest, the metabolic demands of this quiescent muscle are low due to the lack of innervation and hormonal stimulation. Therefore, it may be necessary to increase the metabolic rate of the SOL to detect differences in FA metabolism. We (15) have previously demonstrated
similar results in endurance-trained rat SOL muscle, in which the effects of endurance training on FA oxidation were not detected until the metabolic rate was increased with contraction.

The mechanism by which chronic leptin treatment increases FA oxidation in skeletal muscle is unknown. We hypothesized that leptin would enhance the oxidative potential of skeletal muscle by increasing the activity of key oxidative enzymes CS and β-HAD. Leptin did not alter the activity of these enzymes, demonstrating that increases in FA metabolism are not due to increased oxidative enzyme capacity. A previous study (21) has also failed to demonstrate any change in CS activity of key oxidative enzymes CS and β-HAD. A previous study (21) has also failed to demonstrate any change in CS and β-HAD activity in lean mice after moderate hyperleptinemia. Alternatively, leptin might enhance FA flux into the mitochondrion by 1) increasing the expression of CPT-I or 2) by upregulating its activity, possibly through a reduction in acetyl-CoA carboxylase and, consequently, malonyl-CoA. Evidence for these effects has been demonstrated in pancreatic tissue (41) but not in skeletal muscle. It is also possible that leptin may increase skeletal muscle metabolic rate and FA oxidation through the expression of UCP-3 (23). Clearly, more research is required to understand the mechanisms by which leptin both acutely and chronically enhanced muscle FA metabolism.

Previously (33), we have shown that leptin acutely increases TG hydrolysis. In this set of experiments, we demonstrate that lipolysis is also enhanced after chronic leptin treatment in both resting and contracting SOL. This increase in TG hydrolysis, determined in isolated SOL, is in agreement with the reduced intramuscular TG content measured in SOL muscle in vivo. The enzymatic regulation of TG breakdown in muscle is poorly understood. It is believed that the neutral lipase HSL is the key rate-limiting enzyme for regulating intramuscular TG breakdown (36). We measured total HSL protein expression and found that leptin had no effect on total protein expression. Langfort et al. (22) have previously demonstrated that epinephrine activates HSL by phosphorylation without affecting total enzyme concentration. Thus it is possible that leptin also increases HSL activity by altering its state of phosphorylation.

Although the elevation of serum leptin was associated with numerous significant changes in muscle FA metabolism, we generally failed to note any significant changes in FA metabolism in PF-S animals despite the fact that serum leptin was significantly decreased in this group. However, trends toward increased oxidation and total FA uptake were noted in this group. Although we cannot provide definitive reasons for the absence of significant effects, other factors may have countered the effects of a lower leptin concentration, including other hormonal changes (e.g., increased catecholamines, cortisol), or an altered sensitivity to leptin.

**Potential Role of Insulin**

It has generally been assumed that alterations in metabolism as a consequence of chronic leptin exposure are due directly to the elevated levels of leptin. However, as confirmed in the present study, chronic
leptin administration in the absence of other perturbation (such as diet) may result in the lowering of plasma insulin (20, 31). Because insulin has been shown to stimulate FA uptake and esterification and decrease oxidation and hydrolysis in skeletal muscle (1, 17, 25), it cannot be discounted that a decrease in insulin is at least partly responsible for the observed changes in muscle metabolism. Thus it could be argued that changes in muscle FA metabolism are a secondary, and not a primary, effect of leptin. However, there are two reasons why we believe that the observed metabolic changes are a primary effect of leptin. First, the absolute magnitude of change in leptin [from 1.5 (AD-S) to 9 ng/ml (LEPT)] was much greater than the absolute change in insulin (from 1.36 to 0.18 ng/ml) in the present study. Second, in a recent study by Yaspelkis et al. (39), the injection of leptin for 12–15 days significantly lowered muscle TG and improved insulin responsiveness in rats fed a high-fat diet despite the fact that insulin levels were not significantly lowered. Clearly, without knowing the differences in sensitivity of muscle to insulin and leptin or the threshold concentrations for eliciting a chronic effect, we cannot unequivocally say that the decrease in insulin was without effect. However, for the reasons provided, we think that the increase in leptin is the most important factor.

In summary, we have demonstrated that chronically elevated levels of serum leptin may reduce intramuscular TG in lean rats by stimulating FA oxidation and TG hydrolysis and by reducing total FA uptake. Thus leptin is an important factor in regulating intramuscular TG content in vivo. However, in human obesity, intramuscular TG is elevated despite high levels of circulating leptin (13). We speculate that, in obesity, the development of leptin resistance leads to decreased FA oxidation and TG hydrolysis, resulting in the accumulation of intramuscular TG stores and development of insulin resistance. Research examining the effects of leptin in lean and obese human skeletal muscle FA metabolism is needed.

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