Stimulatory effects of leptin and muscle contraction on fatty acid metabolism are not additive

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Lau, R., W. D. Blinn, A. Bonen, and D. J. Dyck. Stimulatory effects of leptin and muscle contraction on fatty acid metabolism are not additive. Am J Physiol Endocrinol Metab 281:E122–E129, 2001.—Leptin has been shown to acutely stimulate fatty acid oxidation and triacylglycerol hydrolysis in skeletal muscle. These effects are similar to those induced by muscle contraction alone. Several studies have demonstrated that, during aerobic exercise, plasma leptin concentrations are well maintained; however, none has examined whether the stimulatory effects of leptin and contraction on muscle lipid metabolism are additive. This is the first study to examine the direct effect of leptin on lipid and carbohydrate (CHO) metabolism in isolated oxidative muscle over a range of contraction intensities. We examined the effect of leptin (10 μg/ml) on the synthesis and degradation of muscle lipid pools [phospholipid (PL), diacylglycerol (DG), triacylglycerol (TG)] and palmitate oxidation in isolated resting and contracting (2, 8, and 20 tetani/min) soleus muscles. At rest, leptin increased fatty acid oxidation (+40%, P < 0.05) and TG hydrolysis (+47%, P < 0.05), while blunting TG esterification (−20%, P < 0.05). Glucose oxidation was unaffected at rest in the presence of leptin. During tetanic contraction, fatty acid oxidation (+20–114%, P < 0.05) and TG hydrolysis (+19–33%, P < 0.05) as well as net TG utilization (+23%, P < 0.05) were all significantly increased. However, leptin was without further effect on any of these parameters during contraction. Net utilization of intramuscular glycogen, as well as glucose oxidation, was unaffected during contraction by leptin. The findings of the present study indicate that leptin has an important influence on lipid metabolism in resting muscle, but not during contraction.

The hormone leptin, which is produced primarily by adipose tissue, has been shown to regulate food intake and energy expenditure (33). Daily injection with exogenous recombinant leptin in ob/ob mice results in a rapid reduction in food intake and body adiposity (24). Chronic leptin administration has also been demonstrated to reduce triacylglycerol (TG) content in various peripheral tissues such as liver, muscle, and pancreatic cells (30). Acute leptin administration (30–60 min) causes a repartitioning or shunting of fatty acid metabolism toward oxidation and away from esterification in oxidative skeletal muscle. Leptin also stimulates the hydrolysis of stored intramuscular TG (21, 22, 31). The acute effect of leptin on glucose metabolism in muscle has been controversial (5, 16, 28, 34), although there is some evidence of convergence between the leptin- and insulin-signaling pathways in this tissue (2, 22). Thus the direct effects of leptin on skeletal muscle lipid metabolism are well established, whereas the effects of this hormone on glucose metabolism are unclear.

Exercise is well known to increase fatty acid and glucose metabolism. We have previously reported (8) that, in isolated skeletal muscle, electrically induced contraction increases total fatty acid uptake and oxidation. Concomitantly, TG esterification is also increased during contraction (8, 9), but to a much lesser extent than the simultaneous increase in TG hydrolysis. Thus the independent effects of contraction and leptin on skeletal muscle fatty acid oxidation and TG hydrolysis are similar, whereas these metabolic stimuli exert opposing effects on TG esterification. Furthermore, leptin does not acutely increase the total uptake of palmitate in resting muscle (31), as does contraction (8). Collectively, these observations suggest that different signaling pathways can regulate fatty acid metabolism in skeletal muscle. The independent regulation of glucose metabolism by a hormone (insulin) and contraction, for example, is well known. Thus, given that previous observations suggest 1) that leptin and contraction may alter muscle fatty acid metabolism through different mechanisms and 2) that leptin concentrations are generally well maintained in humans (15, 18–20, 25, 27) or increased in rodents (3) during aerobic exercise, it is of considerable importance to establish whether the effects of these two potent stimuli of muscle fatty acid oxidation and TG hydrolysis are additive, analogous to the additive effects of insulin and contraction on glucose metabolism. Furthermore, the effect of leptin on carbohydrate (CHO) metabolism (glucose oxidation, glycogen utilization) during contraction has not previously been examined.

Therefore, we have examined, in isolated soleus muscle, the combined effects of leptin and contraction...
on fatty acid uptake, oxidation, and esterification, as well as on intramuscular TG utilization. In addition, in separate experiments we determined the effects of leptin on glucose oxidation at rest and during contraction, as well as on glycogen utilization. We hypothesized that 1) the leptin- and contraction-induced increases in fatty acid oxidation and TG hydrolysis are additive, 2) there would be no influence of leptin on total fatty acid uptake during contraction, and 3) leptin would blunt the contraction-induced increase in TG esterification. Furthermore, it was our hypothesis that 4) leptin would have no effect on CHO utilization at rest or during muscle contraction. The isolated muscle preparation used in our studies permitted the determination of the independent and combined effects of leptin and muscle contraction on substrate utilization in the absence of other hormonal and systemic perturbations that occur with whole body exercise.

METHODS

Animals

Female Sprague-Dawley rats weighing 180–230 g were used for all experiments. Animals were housed in a controlled environment on a 12:12-h reversed light-dark cycle and fed Purina rat chow ad libitum. All procedures were approved by the animal care committees at the Universities of Guelph and Waterloo. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body mass) before all experimental procedures.

Preparation and Preincubation of Soleus Muscle Strips

Longitudinal muscle strips were prepared without damaging the tendon. 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), gassed (95% O2-5% CO2, pH 7.4) Krebs-Henseleit buffer containing 4% fatty acid-free bovine serum albumin (BSA; Boehringer Mannheim, Laval, QC, Canada), 10 mM glucose, and 1.0 mM palmitate were immediately added to the incubation reservoir. Temperature was maintained at 30°C, and the preincubation medium was continuously gassed for 30 min.

Preexperimental Labeling of the Intramuscular Lipid Pools

Seven milliliters of warmed (30°C), gassed (95% O2-5% CO2, pH 7.4) Krebs-Henseleit buffer containing 4% fatty acid-free BSA, 10 mM glucose, 1.0 mM palmitate, and 2 μCi of [9,10-13C2]palmitate (Amersham Life Science, Oakville, ON, Canada) were immediately added to the incubation reservoir. The incubation medium was continuously gassed, and the temperature was maintained at 30°C by circulating distilled water from a bath through an outer water jacket of the incubation reservoir. Muscles were pulsed with [3H]palmitate for 30 min to prelabel all endogenous lipid pools.

After the pulse, incubation buffer was drained, and muscles were washed for 20 min in the absence of radiolabeled palmitate. After the wash, one-half of the muscles were removed, and lipids were extracted to determine the incorporation of [3H]palmitate. The remaining muscles were incubated for an additional 45 min with [1-13C]palmitate, during which the effects of leptin in resting and contracting soleus were monitored.

Leptin’s Effects on Lipid Metabolism in Resting and Contracting Soleus

Soleus strips were incubated for 45 min, either at rest or stimulated with 150-ms trains comprised of 0.1-ms impulses (20–40 V; 60 Hz) for 45 min at 2, 8, or 20 tetani/min. Within each condition (rest or contraction), muscles were incubated in the presence or absence of a maximally effective dosage (10 μg/ml) of leptin (Amgen, Thousand Oaks, CA). During this experimental phase, exogenous palmitate oxidation and esterification into the intramuscular lipids were monitored with [14C]palmitate, while the rates of intramuscular lipid hydrolysis were simultaneously being monitored by measuring the loss of [3H]palmitate from the lipid (PL, DG, TG) pools. A layer of heavy mineral oil was placed on top of the incubation medium to prevent the escape of 14CO2.

Palmitate Oxidation and Incorporation into Muscle Lipid Pools

Oxidation. Exogenous palmitate oxidation was determined as outlined previously (8, 26), with minor modifications. Gaseous 14CO2 produced during the incubation was determined by transferring a 1.0-ml aliquot of incubation buffer to a sealed glass vial and acidifying with 1.0 ml of 1 M H2SO4. Liberated 14CO2 was captured by a suspended center well containing 1 M benzethonium hydroxide. Center wells were placed in scintillation vials and counted using standard liquid scintillation techniques. Loss of labeled carbon through isotopic exchange (i.e., transfer to acid-soluble metabolites such as acetyl-CoA, etc.) was accounted for by collecting an aliquot of the aqueous phase produced during the muscle lipid extraction for scintillation counting, as we have previously done (8, 26).

Labeled palmitate incorporation into lipid pools. After incubation, muscles were removed, blotted and weighed, and ultrathuraxed in 5.0 ml of ice-cold 1:2 chloroform-methanol solution. Connective tissue remaining on the homogenizing blades was blotted, weighed, and subtracted from the initial wet weight of the muscle. The remainder of the extraction procedure was performed as previously outlined in detail (8, 26). Samples were spotted on silica gel plates (Silica Gel GF 250 μm, Analtech, Newark, DE), and resolved in solvent (60:40:4, heptane-isopropylether-acetic acid) for 45 min. Individual lipid bands were visualized under long-wave ultraviolet light after being sprayed with dichlorofluorescein dye (0.02% wt/vol in ethanol). Samples contained internal standards of phosphatidylcholine, di-, and tripalmitin (Sigma Chemical, St. Louis, MO) to ensure proper lipid identification.

Leptin’s Effects on Intramuscular TG and CHO Utilization

Changes in intramuscular TG and glycogen content as well as glucose oxidation were monitored in a separate set of experiments. For this set of experiments, muscles either remained at rest or were stimulated to contract at 20 tetani/min, because our previous findings had indicated that maximum utilization of intramuscular TG and glycogen occurred at this stimulation frequency (8). Glucose oxidation was determined using 2 μCi of [U-14C]glucose and was monitored by the appearance of 14CO2. Transfer of labeled carbons to acid-soluble metabolites during the oxidation of glucose was accounted for as outlined for the determination of palmitate oxidation.

Muscle glycogen and TG contents were determined on freeze-dried samples, which were dissected free of all visible connective tissue and blood. Glycogen was analyzed in per-
chloric acid (1.0 M) extracts neutralized with 2.2 M KHCO$_3$ (1.0 M EDTA), as described by Harris et al. (14). Muscle TG was extracted from freeze-dried muscle as previously outlined (7, 11).

Calculations and Statistics

The quantity of palmitate esterified and oxidized was calculated from the specific activity of the incubation medium (i.e., ratio of radiolabeled palmitate in dpm to total palmitate in nmol), as outlined previously (8, 9). By use of this ratio, nanomoles of palmitate oxidized to $^{14}$CO$_2$ or incorporated (esterified) into the various intramuscular lipids could be calculated from radioactivity (dpm) measured in the captured CO$_2$ or specific lipid band scraped from the TLC plate. Hydrolysis of intramuscular lipids was calculated as the net loss of preloaded $^3$H label from PL and DG pools and was also calculated on the basis of the specific activity of the incubation medium. The relative partitioning ratio was calculated as the nanomoles of palmitate oxidized divided by the nanomoles of palmitate esterified into TG, as a relative index of the fate of palmitate taken up by the muscle (i.e., oxidation vs. storage as TG). Glucose oxidation was calculated on the basis of the specific activity of labeled glucose in the incubation medium.

Results were analyzed using analysis of variance (ANOVA) procedures, and a Fisher’s protected least significant differences post hoc test was used to test significant differences revealed by the ANOVA. Significance was accepted at $P < 0.05$. All data are reported as means ± SE.

RESULTS

Palmitate Uptake, Oxidation, and Esterification at Rest and During Contraction

Muscle contraction resulted in increased oxidation of palmitate (Fig. 1A), ranging from 20% at 2 tetani/min ($P > 0.05$) to 48% at 8 tetani/min ($P < 0.05$) and 114% at 20 tetani/min ($P < 0.05$). Leptin significantly increased palmitate oxidation in resting muscle (+40%; $P < 0.05$), but its effects were not additive to that of contraction (Fig. 1A).

Neither leptin nor contraction had any effect on esterification of fatty acids into PL or DG pools (Table 1). There was, however, a significant decrease in TG esterification at rest (20%, $P < 0.05$) in the presence of leptin (Fig. 1B). Contraction significantly enhanced the rate of TG esterification at 8 and 20 tetani/min by 19 and 33%, respectively ($P < 0.05$). The increase in TG esterification during contraction was unaffected at all tetanic frequencies in the presence of leptin (Fig. 1B).

Total palmitate uptake was calculated as the sum of the amount oxidized and esterified into all intramuscular lipids (PL, DG, and TG). Total palmitate uptake increased linearly as a function of contraction frequency and was not altered in the presence of leptin (Fig. 2). The relationship between the absolute rates of palmitate oxidized and palmitate esterified to TG has been shown to be highly linear (8). A similar relationship between oxidation and esterification was also observed in the present study (Fig. 3A) except during the resting condition in the presence of leptin. The partitioning, or shunting, of fatty acids toward oxidation and away from TG esterification was significantly elevated at rest in the presence of leptin (0.83 ± 0.07 vs. 0.49 ± 0.03; $P < 0.05$; Fig. 3B). This was not due to an alteration in total palmitate uptake by leptin. During contraction, as total palmitate uptake increased, shunting toward fatty acid oxidation also increased and was not altered by the presence of leptin (Fig. 3B).

Effects of Leptin and Contraction on Intramuscular Lipid Utilization and CHO Metabolism

Intramuscular TG hydrolysis has previously been shown to be the major source of aerobic ATP provision in intensely contracting soleus muscle (8). Leptin caused a significant decrease in intramuscular TG content at rest during 45 min of incubation (~43%; Fig. 4), in agreement with a greater loss of $[^3]$Hpalmitate from this pool in the presence of leptin (40.0 ± 1.5 vs. 17.8 ± 5.5 nmol/g, $P < 0.05$). Contraction alone resulted in a significant decrease in intramuscular TG (~24%, $P < 0.05$) but was not enhanced in the presence of leptin (Fig. 4). Leptin was also without effect on PL or DG hydrolysis (Table 2).
Glucose oxidation increased significantly (+66%; \( P < 0.05 \)) from rest to contraction (20 tetani/min) but was unaffected by leptin (Table 3). Muscle glycogen content decreased only marginally (−11%; \( P > 0.05 \)) when muscles were stimulated to contract at 20 tetani/min (Table 3). This decrease was unaffected by leptin.

**DISCUSSION**

Leptin and contraction both profoundly alter fatty acid metabolism in muscle. The independent effects of leptin and contraction each result in the stimulation of fatty acid oxidation and TG hydrolysis. However, whereas contraction also increases total fatty acid uptake and TG esterification, leptin has little effect on uptake and decreases TG esterification. Collectively, these findings suggest that different signaling pathways may be involved in the regulation of fatty acid metabolism by leptin and contraction. Thus, given that leptin and contraction may independently alter muscle fatty acid metabolism through different signaling mechanisms and that leptin concentrations are generally well maintained during aerobic exercise, it is important to determine whether the effects of these two potent stimuli are additive. However, no study has examined the impact of leptin on muscle substrate utilization during contraction.

Therefore, we have examined the effects of leptin and contraction on fatty acid and CHO metabolism in an isolated soleus muscle preparation in the absence of confounding systemic perturbations that occur during whole body exercise. In this study, we have confirmed previous observations that, in resting muscle, leptin

<table>
<thead>
<tr>
<th>Lipid Pool</th>
<th>Rest 2 TPM</th>
<th>Control</th>
<th>Leptin</th>
<th>Rest 8 TPM</th>
<th>Control</th>
<th>Leptin</th>
<th>Rest 20 TPM</th>
<th>Control</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>20.9 ± 3.3</td>
<td>24.7 ± 3.3</td>
<td>34.4 ± 1.8</td>
<td>35.4 ± 3.0</td>
<td>33.3 ± 4.7</td>
<td>32.9 ± 4.0</td>
<td>37.5 ± 6.1</td>
<td>37.3 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>DG</td>
<td>27.2 ± 6.8</td>
<td>33.9 ± 7.5</td>
<td>40.2 ± 6.8</td>
<td>33.8 ± 5.1</td>
<td>44.1 ± 7.0</td>
<td>39.5 ± 7.7</td>
<td>33.1 ± 5.9</td>
<td>32.2 ± 10.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, expressed in nmol/g wet wt; \( n = 6–7 \) animals per treatment. PL, phospholipid; DG, diacylglycerol; TPM, tetani per minute.

**Fig. 2.** Effect of contraction on total palmitate uptake in soleus in the presence and absence of leptin.

**Fig. 3.** Effect of leptin on the linear relationship between TG esterification and palmitate oxidation (A) and relative partitioning of fatty acids toward oxidation and away from storage (oxidation/TG esterification; B). a) significantly different from the no-leptin group; *significantly different from rest.
acutely partitions fatty acids toward oxidation and away from TG esterification and increases the rate of lipolysis. Furthermore, our findings indicate that the independent effects of leptin are completely eliminated during contraction even at low rates of stimulation frequency (i.e., 2 tetani/min). Leptin was without effect on CHO metabolism either at rest or during contraction. It should be noted, however, that the findings of the present study are specific to the isolated muscle preparation utilized. It is possible that, in the intact organism, there may be some significant interaction between leptin and other hormones such as insulin, catecholamines, and the like, that may influence TG turnover or fatty acid metabolism.

**Effect of Leptin on Lipid Metabolism at Rest**

In this study, we have demonstrated that, in resting muscle, a maximally stimulating dosage of leptin causes a 40% increase in palmitate oxidation and a 20% decrease in TG esterification, results which are similar to the previous findings of Muoio and colleagues (21, 22) and Steinberg and Dyck (31). In addition, leptin acutely enhanced the rate of TG hydrolysis, as shown by the enhanced loss of $^{3}$H label from the TG pool and the 43% decrease in intramuscular TG content. However, the mechanisms for leptin’s effect on resting muscle fatty acid metabolism are unknown. Because leptin did not affect total palmitate uptake in the present study, the increase in oxidation does not appear to be secondary to altered fatty acid transport across the sarcolemma.

It is more likely, therefore, that leptin regulates fatty acid oxidation at a critical site downstream of transport, such as at carnitine palmitoyltransferase I (CPT-I), which regulates the flux of fatty acids across the inner mitochondrial membrane. Exposure to leptin for 48 h increases fatty acid oxidation in $\beta$-islet cells, which coincides with the enhanced expression of CPT-I and decreased expression of acetyl-CoA carboxylase (33). Acetyl-CoA carboxylase catalyzes the formation of malonyl-CoA, which is a potent inhibitor of CPT-I and fatty acid oxidation in isolated rodent muscle (1). However, the effects of leptin on the expression of these enzymes have not been examined in skeletal muscle and may not explain the acute (minutes) regulation of fatty acid metabolism observed in the present study, a time frame that would presumably not allow for these changes. Thus, whether leptin acutely downregulates the production of malonyl-CoA or alters the sensitivity of CPT-I to this inhibitor in muscle warrants examination. It is noteworthy, however, that insulin, which opposes the acute effects of leptin on fatty acid oxidation and esterification (i.e., $\leq$90 min) increases the content of malonyl-CoA in muscle (1).

Another potential mechanism by which leptin might stimulate fatty acid metabolism in muscle is via the regulation of the recently discovered uncoupling protein 3 (UCP3), which is expressed preferentially in this tissue (12). UCPs have been implicated in mediating leptin’s thermogenic effects, and the expression of UCP3 has been shown to be upregulated by leptin within days (12). There is now recent evidence that UCP3 may serve primarily as a regulator of lipid metabolism in muscle and can be upregulated at both the message and protein levels within 30 min (32). It is tempting, therefore, to speculate that one of the mechanisms by which leptin might acutely upregulate fatty acid metabolism in muscle is through a rapid increase in UCP3 expression. Thus it is possible that leptin may mediate its stimulatory effects on fatty acid oxidation through both a simultaneous reduction in CPT-I inhibition.

### Table 2. Effect of leptin on PL and DG hydrolysis at rest and during various rates of muscle contraction

<table>
<thead>
<tr>
<th>Lipid Pool</th>
<th>Rest</th>
<th>2 TPM</th>
<th>8 TPM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Leptin</td>
<td>Control</td>
</tr>
<tr>
<td>PL</td>
<td>2.8 ± 0.6</td>
<td>1.8 ± 0.7</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>DG</td>
<td>4.3 ± 2.0</td>
<td>4.1 ± 1.2</td>
<td>4.5 ± 1.6</td>
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</table>

Values are means ± SE, expressed in nmol/g wet wt; n = 6–7 animals per treatment.
bition and an enhanced proton/fatty acid leak across the inner mitochondrial membrane.

Effect of Leptin on Lipid Metabolism During Contraction

The primary objective of this study was to determine whether the independent stimulatory effects of contraction and leptin on fatty acid oxidation and TG hydrolysis would be additive and whether leptin would blunt the contraction-induced increase in TG esterification. Our results clearly demonstrate that leptin does not alter the effects of contraction on fatty acid metabolism. In agreement with our previous findings (8), palmitate oxidation continued to increase linearly with increasing contraction frequency, but no additive effect of leptin was observed. In fact, our findings indicate that the stimulation of fatty acid oxidation by leptin is not additive to contraction, even at low rates of contraction frequency (i.e., 2 tetani/min), when the stimulation of oxidation is actually less than that induced by leptin alone. These findings strongly suggest the existence of a divergence of leptin- and contraction-signaling pathways and that stimulation of the contraction pathway inhibits the signaling cascade initiated by leptin. Thus the contraction-mediated increases in lipid metabolism appear to be most strongly influenced by the energy demands of the cell. Activation of AMP kinase (AMPK) during contraction inhibits acetyl-CoA carboxylase and malonyl-CoA production (29) and may be a primary signal involved in the upregulation of muscle fatty acid metabolism. The activation of AMPK has recently been shown to result in the simultaneous stimulation of fatty acid oxidation and inhibition of TG esterification, causing a repartitioning of fatty acids away from storage (23), an effect which has been observed in this and other studies at rest in the presence of leptin (21, 22). However, during contraction, when there is also a relative shunting of fatty acids toward oxidation, the rate of TG esterification is actually increased. Because this effect would not be predicted by the stimulation of AMPK, it is probable that the stimulation of fatty acid metabolism during muscle contraction involves other factors as well.

Acute Effect of Leptin on CHO Metabolism

The effects of leptin on CHO metabolism in skeletal muscle, despite having been studied more extensively, are much less clear than the effects of leptin on fatty acid metabolism. The hypothesized role of leptin in regulating CHO metabolism was initially derived from studies documenting a normalization of serum glucose levels and insulin sensitivity in obese mice after chronic leptin administration (24). Although some evidence exists for cross talk between the leptin- and insulin-signaling pathways (17), there is considerable controversy regarding the acute effects of leptin on glucose uptake and oxidation. Some studies have demonstrated that leptin acutely upregulates glucose uptake (5, 6, 13, 16), whereas others have failed to observe any acute effect (4, 21, 34). It has been suggested that an intact sympathetic nervous system may be a prerequisite for leptin to exert an acute effect on glucose uptake. This is supported by the failure to elicit a stimulatory effect on glucose uptake when either the muscle is denervated (16) or norepinephrine release is blocked (13). Our findings are in agreement with those studies that fail to support the hypothesis that leptin directly stimulates glucose uptake and metabolism. The maximal dosage of leptin used in this study failed to enhance glucose oxidation either at rest or during intense contraction (20 tetani/min). Leptin also failed to alter soleus glycogen utilization during contraction, although it should be noted that the decrease in glycogen content during contraction was small. To more definitively test leptin’s effect on glycogen utilization, it would be desirable to repeat these experiments with use of the extensor digitorum longus muscle, which relies more extensively on glycogen metabolism than the soleus during contraction.

Physiological Role of Leptin During Exercise?

This is the first study to examine the interaction of leptin and contraction on substrate utilization in contracting skeletal muscle. A great deal of evidence has accumulated regarding the effect of aerobic exercise on circulating levels. In this study, we present evidence that leptin does not play a direct role in regulating substrate utilization in contracting muscle. Interestingly, a recent study by Essig et al. (10) demonstrated that leptin concentrations were not significantly decreased by aerobic exercise until 48 h into recovery. This may suggest a role for leptin during the recovery from exercise, i.e., that a decrease in circulating leptin may stimulate appetite, reduce metabolic rate, and permit a relative partitioning of lipids toward storage (10) to allow for the restoration of the body’s energy reserves.

Table 3. Effect of leptin on glucose oxidation and glycogen utilization after 45 min of incubation at rest or contraction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rest</th>
<th>Leptin</th>
</tr>
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<tbody>
<tr>
<td>I[U-14C]glucose oxidation</td>
<td>759.4 ± 157.5</td>
<td>846.8 ± 157.5</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>116.5 ± 6.1</td>
<td>119.3 ± 8.3</td>
</tr>
<tr>
<td>20 TPM</td>
<td>Control</td>
<td>Leptin</td>
</tr>
<tr>
<td>I[U-14C]glucose oxidation</td>
<td>1,270.2 ± 157.5*</td>
<td>1,404.9 ± 143.8*</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>101.0 ± 4.2</td>
<td>105.6 ± 3.9</td>
</tr>
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</table>

Values are means ± SE, expressed in nmol/g wet wt; n = 6–8 animals per treatment. *Significantly different from rest.
In summary, an isolated soleus muscle preparation was utilized to directly examine the effect of leptin on muscle substrate utilization at rest and over a range of contraction frequencies (2–20 tetani/min). At rest, leptin caused a repartitioning of incorporated fatty acids toward oxidation and away from esterification and significantly enhanced the absolute and net rates of TG hydrolysis. These effects were completely eliminated at all contraction frequencies, which independently altered muscle lipid metabolism in a fashion similar to that observed at rest in the presence of leptin. Thus, contraction's stimulatory effects on fatty acid metabolism (oxidation, hydrolysis, esterification) appear to be linked to the energy demands of the cell and completely override the leptin-signaling pathway. Leptin was without effect on CHO metabolism at rest and during contraction. These findings suggest that leptin's metabolic role on muscle substrate utilization becomes insignificant during contraction.

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