Leptin opposes insulin’s effects on fatty acid partitioning in muscles isolated from obese ob/ob mice

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Muoio, Deborah M., G. Lynis Dohm, Edward B. Tapscott, and Rosalind A. Coleman. Leptin opposes insulin's effects on fatty acid partitioning in muscles isolated from obese ob/ob mice. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E913–E921, 1999.—Because muscle triacylglycerol (TAG) accumulation might contribute to insulin resistance in leptin-deficient ob/ob mice, we studied the acute (60- to 90-min) effects of leptin and insulin on [14C]glucose and [14C]oleate metabolism in muscles isolated from lean and obese ob/ob mice. In ob/ob soleus, leptin decreased glycogen synthesis 36–46% (P < 0.05), increased oleate oxidation 26% (P < 0.05), decreased oleate incorporation into TAG 32% (P < 0.05), and decreased the oleate partitioning ratio (oleate partitioned into TAG/CO2) 44% (P < 0.05). Insulin decreased oleate oxidation 31% (P < 0.05), increased oleate incorporation into TAG 46% (P < 0.05), and increased the partitioning ratio 125% (P < 0.01). Adding leptin diminished insulin's antioxidative, lipogenic effects. In soleus from lean mice, insulin increased the partitioning ratio 142%, whereas leptin decreased it 51%, as previously reported (Muoio, D. M., G. L. Dohm, F. T. Friedorek, E. B. Tapscott, and R. A. Coleman. Diabetes 46: 1360–1363, 1997). The phosphatidylinositol 3-kinase inhibitor wortmannin blocked insulin's effects on lipid metabolism but only attenuated leptin's effects. Increasing glucose concentration from 5 to 10 mM did not affect TAG synthesis, suggesting that insulin-induced lipogenesis is independent of increased glucose uptake. These data indicate that leptin opposes insulin’s promotion of TAG accumulation in lean and ob/ob muscles. Because acute leptin exposure does not correct insulin resistance in ob/ob mice, in vivo improvements in glucose homeostasis appear to require other long-term factors, possibly TAG depletion.

fatty acid oxidation; obesity; triacylglycerol

LEPTIN, a 16-kDa peptide encoded by the ob gene, is the first identified adipocyte-derived hormone that directly regulates adiposity and energy homeostasis by decreasing food intake and increasing energy expenditure (30, 47). Homozygous ob/ob mice, which lack functional leptin, are characterized by severe adiposity, hyperlipidemia, hyperglycemia, and hyperinsulinemia (47), thereby exhibiting an obesity syndrome that is phenotypically similar to type II diabetes. When administered to ob/ob mice, leptin reverses obesity and normalizes serum concentrations of glucose, insulin, and lipids (30, 39). Leptin affects these serum variables at low doses that do not affect body weight (30), and improvements in serum variables are greater in leptin-treated ob/ob mice than in pair-fed ob/ob controls (22). These observations indicate that leptin exerts metabolic effects that are independent of its effects on food intake and body weight.

Leptin receptors (L-Rs) are expressed primarily in the hypothalamus but are also present in several peripheral tissues, including liver, pancreas, ovary, kidney, heart, and skeletal muscle (5, 13), suggesting that leptin may exert direct effects on tissues other than the brain. Emerging evidence indicates that peripheral L-Rs play an important physiological role in mediating leptin’s regulation of fuel homeostasis. We reported that leptin controls skeletal muscle lipid metabolism by partitioning fatty acids (FA) toward oxidation and away from storage as triacylglycerol (TAG; see Ref. 26). In isolated soleus muscle from lean C57BL/6J mice, leptin acutely increases oleate oxidation and decreases oleate incorporation into TAG. In addition, we reported that leptin attenuates insulin’s antioxidative and lipogenic effects on skeletal muscle lipid utilization. Our data from acute incubations of skeletal muscle are similar to those obtained from chronically incubated isolated pancreatic islets in which 3 days of leptin exposure increased FA oxidation, decreased FA esterification, and depleted islet cell TAG content (40). These data demonstrate that leptin directly regulates peripheral lipid metabolism.

Skeletal muscle is a major determinant of whole body glucose and lipid metabolism and is the primary tissue accountable for reduced whole body insulin responsiveness in type II diabetes. The pathological mechanisms underlying type II diabetes are still largely undefined, but considerable evidence links the development of muscle insulin resistance with increased muscle lipid content (28, 31). Because in vivo leptin treatment dramatically improves insulin sensitivity and depletes muscle TAG in ob/ob mice (22, 30, 40), we studied the acute effects of leptin and its interaction with insulin on glucose metabolism and FA partitioning in muscles isolated from ob/ob mice. Contrary to our hypothesis that acute leptin exposure would improve insulin-mediated glucose regulation, we found that leptin decreased glycogen synthesis in muscles from ob/ob mice. We also report that, in muscles isolated from ob/ob mice, insulin promotes TAG accumulation, whereas leptin favors lipid oxidation and TAG depletion. These data indicate that leptin and insulin have acute opposing effects on fuel metabolism in muscles from ob/ob mice and suggest that leptin-induced im-

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provements in glucose homeostasis observed in vivo may require centrally mediated effects and/or long-term regulatory factors such as protein synthesis and muscle TAG depletion.

RESEARCH DESIGN AND METHODS

Animals. Female C57BL/6j ob/ob mice and lean littermates (Jackson Laboratory, Bar Harbor, ME) were maintained on a 12:12-h light-dark cycle with food (Purina mouse chow) and H2O available ad libitum. During the studies of glucose metabolism, Purina chow was removed from the animal's cages at 1700 on the evening before the experiments. Animals used for glucose experiments were 8–12 wk old because leptin improves glucose homeostasis in ob/ob mice of this age (30, 39). Because older animals are preferred for studying lipid metabolism, animals used for lipid experiments were 12–18 wk old. The animal protocol was approved by the University of North Carolina Institutional Animal Care and Use Committee.

Muscle incubations. Recombinant murine ob peptide (leptin) was supplied by Amgen (Thousand Oaks, CA). Soleus and extensor digitorum longus (EDL) muscles were removed from 0900 and 1100 under anesthesia (100 mg/kg ketamine and 10 mg/kg xylazine), cleaned free of adipose and connective tissue, and immediately transferred to a 24-well tissue culture dish in a shaking H2O bath at 29°C. Each well contained 1.0 ml of a modified Krebs-Ringer bicarbonate buffer (KRB; low-calcium Krebs Henseleit bicarbonate buffer (8), 1.0% (wt/vol) diazyl, FA-free BSA, and 5.0 mM glucose) and was gassed continuously with 95% O2-5% CO2. After all muscles were isolated, they were preincubated for an additional 15 min at 37°C before being transferred to fresh media (as will be described).

Lipid metabolism. In experiments that examined hormone effects on lipid partitioning, muscles were preincubated for an additional 20 min at 37°C in KRB media with 1.0 mM oleate, 1.0 mM carnitine, and 1.5% BSA (KRB + FA). Muscles were then transferred to fresh KRB + FA containing (1-14C)oleate (1.0 µCi/ml; American Radio Chemical, St. Louis, MO) and incubated for 90 min. Previous dose-response experiments indicated that, in isolated muscles, maximal hormone responses are obtained at 10.0 mM insulin and 10.0 µg/ml leptin (12, 18, 26). Maximal levels of hormone, either insulin (Eli Lilly, Indianapolis, IN) alone (10.0 µM insulin), or insulin and leptin combined, were added to one-half of the muscles, and the contralateral soleus and EDL muscles from each mouse were used as basal (non-hormone-treated) controls. Immediately after the incubation, semidry filter paper (Whatman no. 3) saturated with 2 N NaOH was placed over the 24-well incubation dish and tightly covered with a foam pad and the plate cover. [14C]CO2 produced by muscle was trapped by liquid scintillation counting. Increases in 14C over background were minimal in media in which muscles had been incubated, indicating that insignificant amounts of acid-soluble metabolites had been released in the medium (14). Lipids were extracted from muscle, and TAG, diacylglycerol (DAG), phospholipid, and FA were separated by thin-layer chromatography on scored silica gel G plates (Fisher Scientific, Norcross, GA). Lipids were extracted in hexane-diethyl ether-acetic acid (80:20:1, vol/vol/vol). Radioactivity incorporation into different lipid species was determined by comparison with standards using a Bioscan 200 system.

Carbohydrate metabolism. In experiments that examined hormone effects on glucose metabolism, muscles were preincubated for an additional 20 min at 37°C in KRB, transferred to fresh KRB containing [U-14C]glucose (1.0 µCi/ml; Sigma Chemical, St. Louis, MO), and incubated for 60 min. Maximal levels of hormone, either insulin alone (10.0 µM insulin), leptin alone (10.0 µg/ml), or insulin and leptin combined, were added to one-half of the muscles, and the contralateral soleus and EDL muscles from each mouse were used as basal (non-hormone-treated) controls. Immediately after the incubation, semidry filter paper (Whatman no. 3) saturated with 2 N NaOH was placed over the 24-well incubation dish, and [14C]CO2 produced by muscle was trapped and quantified as described in Lipid metabolism as an indicator of glucose oxidation. After the CO2 trapping procedure, muscles were removed from the wells, weighed, and extracted in KOH to precipitate glycogen (12). Radioactivity incorporated into glycogen was measured by liquid scintillation counting. Lactate concentrations in neutralized medium were assayed enzymatically (9).

Statistics. Data are presented as means ± SE and were analyzed by a one-way ANOVA followed by comparison using Newman-Keuls post hoc test. Because muscles from ob/ob mice exhibited significant interanimal variation in basal fuel metabolism as well as in hormone responsiveness, differences between basal and hormone-treated intralateral muscles were also analyzed by paired Student's t-test.

RESULTS

Leptin inhibits glycogen synthesis. Body weights of obese ob/ob mice (53 ± 7.4 g) were two times those of lean littermates (25 ± 3.8 g). In soleus and EDL muscles from lean animals, insulin increased glycogen synthesis by eight- and sixfold, respectively (Fig. 1A). Compared with muscles from lean mice, insulin-stimulated increases in glycogen synthesis were ~50% lower in muscles from ob/ob mice (P < 0.01), consistent with obesity-associated insulin resistance. In soleus muscles from ob/ob mice, leptin inhibited glycogen synthesis by 35% (P < 0.05; Fig. 1B); adding insulin abolished leptin's inhibition. In EDL, leptin diminished
Leptin and insulin regulate esterification of FA into TAG in ob/ob muscles. Under basal conditions the rates of FA incorporation into TAG were similar in muscles from ob/ob and lean mice (Table 1). Hormone effects on FA esterification were opposite of their effects on FA oxidation (Fig. 2B). In soleus muscle, insulin stimulated the esterification of FA into TAG by 47% \( (P < 0.01) \), whereas leptin decreased FA incorporation into TAG by 32% \( (P < 0.05) \). In EDL, neither leptin nor insulin affected incorporation into TAG.

Leptin and insulin have opposing effects on muscle lipid partitioning in ob/ob muscles. FA entering muscle can be esterified and incorporated into complex lipids (primarily TAG) to provide the muscle with an endogenous energy reservoir, or FA can be oxidized to provide an immediate source of energy. The partitioning of FA between these two fates largely determines the TAG content of muscle. To quantify the partitioning of FA between biosynthetic and oxidative pathways, we divided the nanomoles per gram muscle per hour of FA esterified into TAG by the nanomoles per gram muscle per hour of FA oxidized to CO\(_2\), providing a ratio of lipid stored to lipid oxidized (Fig. 3). In soleus and EDL muscles the ratio of FA stored to lipid oxidized was ∼4:1 and 2:1, respectively, under basal conditions. The divergent effects of each hormone on FA oxidation compared with FA storage resulted in marked changes in muscle lipid partitioning. In soleus muscle, insulin increased this ratio by 125%, whereas leptin decreased it by 44% \( (P < 0.01 \text{ and } P < 0.05) \). When muscles were coincubated with both hormones, leptin blocked the insulin-induced increase in the ratio of FA incorporated into TAG/CO\(_2\). EDL exhibited similar, but less pronounced, hormone-mediated changes in FA partitioning. These data indicate that, in muscles from ob/ob mice, insulin promotes TAG accumulation, whereas leptin favors TAG depletion.

Insulin affects FA incorporation into other complex lipids. In muscles isolated from lean mice, insulin increased \(^{14}\text{C}\) label incorporation into DAG by 42% \( (P < 0.05) \), respectively. These data differ from our previous observations using muscles from lean mice in which leptin did not alter basal or insulin-stimulated glycolysis synthesis (26). Similar to muscles from lean mice (26), leptin did not affect glucose oxidation or lactate production in ob/ob muscles incubated with or without insulin (data not shown).

Leptin and insulin regulate FA oxidation in ob/ob muscles. Basal rates of FA oxidation in ob/ob muscles did not differ from those in muscles from lean mice (Table 1). Compared with contralateral basal controls, insulin inhibited the rate of FA oxidation in soleus by 31% and in EDL by 23% \( (P < 0.05) \). In contrast, leptin increased the rates of FA oxidation in both soleus and EDL muscles by 26 and 20%, respectively \( (P < 0.05) \). When leptin and insulin were present together, leptin completely blocked insulin’s antioxidative effect.

| Table 1. Basal rates of oleate oxidation and esterification into TAG in muscles from ob/ob mice and lean littermates |
|---|---|---|---|
| | Soleus | | EDL |
| Olate oxidized to CO\(_2\) | 179 ± 36\(^*\) | 188 ± 10 | 168 ± 27\(^*\) | 179 ± 14 |
| Olate esterified in TAG | 909 ± 30\(^*\) | 762 ± 72 | 303 ± 16\(^*\) | 306 ± 32 |

Data are expressed as means ± SE; \( n = 15 \) muscles/group. Units are mmol oleate·g muscle\(^{-1}\)·h\(^{-1}\). Intact soleus and extensor digitorum longus (EDL) muscles from ob/ob mice and lean littermates were incubated in 1.0 ml of modified Krebs-Ringer media containing 1.5 mM oleate and 5.0 mM glucose. Soleus and EDL muscles were incubated for 90 min at 37°C in the presence of 1.0 \( \mu \text{Ci/ml} \). [\(^{14}\text{C}\)]Oleate. Olate oxidation to CO\(_2\) and incorporation into muscle triacylglycerol (TAG) were determined as described in RESEARCH DESIGN AND METHODS. Data were analyzed by unpaired Student’s t-test. * Data from reference (26) show comparisons between muscles from lean and ob/ob mice.
However, in soleus and EDL from ob/ob mice, insulin increased the percentage of FA incorporated into DAG (P < 0.05). In soleus from both lean and ob/ob mice, insulin decreased the proportion of label incorporated into phospholipid (P < 0.05). Adding leptin to the media did not alter the fractions of FA label incorporated into different lipid species, nor did it affect the absolute amount of labeled DAG (data not shown).

Insulin's effects on FA partitioning are mediated by phosphatidylinositol 3-kinase. Insulin-mediated regulation of muscle glucose metabolism requires activation of phosphatidylinositol 3-kinase (PI 3-kinase; see Refs. 21 and 46). PI 3-kinase may also be involved in leptin signaling (6). To determine whether PI 3-kinase mediates insulin and/or leptin-induced alterations in muscle lipid partitioning, we incubated solei from wild-type animals with insulin or leptin, alone or in the presence

Fig. 2. Effects of leptin and insulin on muscle fatty acid (FA) oxidation and FA incorporation in triacylglycerol (TAG). Intact soleus and EDL muscles from ob/ob mice were incubated in 1.0 ml of modified Krebs-Ringer media containing 1.5 mM oleate and 5.0 mM glucose. Then, one muscle from each animal was used as a basal (non-hormone-treated) control (n = 15 muscles), and the contralateral soleus and EDL muscles (n = 5/group) were incubated for 90 min at 37°C in the presence of either insulin (10.0 mU/ml), leptin (10.0 µg/ml), or insulin plus leptin. Incubation media contained 1.0 µCi/ml of [1-14C]oleate. [14C]oleate incorporation into CO2 (A) and muscle TAG (B) was determined as described in RESEARCH DESIGN AND METHODS. Data (means ± SE) are expressed as percent change in hormone-treated muscles compared with nontreated contralateral controls (basal values are provided in text). Data were analyzed by paired Student's t-test. *P < 0.05 and **P < 0.01, statistically significant differences compared with basal values.

Fig. 3. Effects of leptin and insulin on muscle FA partitioning. Intact soleus (A) and EDL (B) muscles from ob/ob mice were incubated in 1.0 ml of modified Krebs-Ringer media containing 1.5 mM oleate and 5.0 mM glucose. Then, one muscle from each animal was used as a basal (non-hormone-treated) control (n = 15 muscles), and the contralateral soleus and EDL muscles (n = 5/group) were incubated for 90 min at 37°C in the presence of either insulin (10.0 mU/ml), leptin (10.0 µg/ml), or insulin plus leptin. Incubation media contained 1.0 µCi/ml of [1-14C]oleate. Label incorporation into CO2 was determined as described in RESEARCH DESIGN AND METHODS. Data (means ± SE) are expressed as a ratio of [14C]oleate incorporated into TAG (nmol·g⁻¹·h⁻¹) divided by label incorporated into CO2 (nmol·g⁻¹·h⁻¹). Data were analyzed by one-way ANOVA. *P < 0.05 and **P < 0.01, significant differences between basal and hormone-treated muscles; ‡P < 0.05, differences between insulin alone and other hormone treatments.
METHODS. Totals (nmol oleate·g muscle
were incubated for 90 min at 37°C in the presence of insulin (10.0 mU/ml) (preincubation, one muscle from each animal was used as a basal (non-hormone-treated) control, and the contralateral soleus and EDL muscles
Effects of insulin on incorporation of FA into different lipid species
Table 2.

RESEARCH DESIGN AND METHODS, and data are presented as means
Fig. 4. Effects of insulin on muscle FA incorporation into diacylglycerol (DAG). Intact soleus and EDL muscles from lean (A) and ob/ob (B) mice were incubated in 1.0 ml of modified Krebs-Ringer media containing 1.5 mM oleate and 5.0 mM glucose. Then, one muscle from each animal was used as a basal (non-hormone-treated) control (n = 5), and the contralateral soleus and EDL muscles (n = 5) were incubated for 90 min at 37°C in the presence of insulin (10.0 µU/ml). Incubation media contained 1.0 µCi/ml of [1-14C]oleate. 14C label incorporation into muscle DAG was determined as described in RESEARCH DESIGN AND METHODS, and data are presented as means ± SE. *P < 0.05 and **P < 0.01, significant differences between basal and contralateral insulin-treated muscles by paired Student's t-test. †P < 0.05, comparisons between lean and ob/ob muscles by unpaired Student's t-test.

Glucose competes with FA as a substrate for oxidation and, upon entering the glycolytic pathway, provides glycerol 3-phosphate for de novo glycerolipid biosynthesis. Thus, because wortmannin prevents insulin-stimulated glucose uptake (21, 46), wortmannin's inhibition of insulin-mediated FA partitioning (Fig. 5, A-C) might suggest that insulin's effects are secondary to increased glucose uptake. To test the effect of glucose uptake directly, we incubated muscles from lean mice with and without insulin in the presence of increasing glucose concentrations to increase insulin-independent glucose uptake. In the absence of insulin, increasing the glucose concentration from 5 to 10 mM decreased FA oxidation by 24 and 28% (P < 0.05) in soleus and EDL muscles, respectively, but had no effect on FAA incorporation into TAG (Fig. 6). Adding insulin decreased FA oxidation by 37–40% and increased FA incorporation into TAG by 57–66% in soleus and EDL muscles. Doubling the media glucose concentration from 5 to 10 mM did not, however, augment insulin's antioxidative or lipogenic effects. These data suggest that an insulin-mediated increase in glucose uptake could inhibit muscle FA oxidation but is not responsible for the increase in FA esterification.

DISCUSSION

In isolated soleus and EDL muscles from ob/ob mice, leptin and insulin had acute and opposing effects on muscle lipid metabolism; leptin favored lipid oxidation, whereas insulin favored muscle lipid storage as TAG.

Table 2. Effects of insulin on incorporation of FA into different lipid species

<table>
<thead>
<tr>
<th>Lipoide</th>
<th>TAG (nmol/g muscle/h)</th>
<th>DAG (nmol/g muscle/h)</th>
<th>PL (nmol/g muscle/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Insulin</td>
<td>Basal</td>
</tr>
<tr>
<td>Soleus (+/−)</td>
<td>85.2 ± 2.5</td>
<td>87.0 ± 2.3</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>EDL (+/−)</td>
<td>72.0 ± 4.9</td>
<td>75.0 ± 3.6</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td>Soleus (ob/ob)</td>
<td>80.9 ± 3.1</td>
<td>79.7 ± 3.3</td>
<td>7.6 ± 1.4</td>
</tr>
<tr>
<td>EDL (ob/ob)</td>
<td>72.2 ± 4.2</td>
<td>68.4 ± 5.2</td>
<td>9.3 ± 1.1</td>
</tr>
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</table>

Data are means ± SE and are expressed as the percentage of total label recovered from muscle lipids. Intact soleus and EDL muscles from ob/ob mice and lean littersmates were incubated in 1.0 ml of modified Krebs-Ringer media containing 1.5 mM oleate and 5.0 mM glucose. After preincubation, one muscle from each animal was used as a basal (non-hormone-treated) control, and the contralateral soleus and EDL muscles were incubated for 90 min at 37°C in the presence of insulin (10.0 µU/ml) (n = 5/group). Media contained 1.0 µCi/ml of [1-14C]oleate, and [14C]oleate incorporation into muscle TAG, diacylglycerol (DAG), and phospholipid (PL) was determined as described in RESEARCH DESIGN AND METHODS. Totals (nmol oleate·g muscle−1·h−1) were soleus (+/−), 1,067 ± 117; EDL (+/−), 421 ± 96; soleus (ob/ob), 897 ± 89; EDL (ob/ob), 424 ± 44. *P < 0.05, significant differences between basal and contralateral insulin-treated muscles by paired Student's t-test. †Comparisons between lean and ob/ob muscles by unpaired Student's t-test.
Consistent with our previous report on leptin’s effects on muscles from lean mice (26), hormone-induced alterations in muscle lipid metabolism were more pronounced in soleus, a highly oxidative muscle, than EDL, a more glycolytic muscle.

Our data provide additional evidence that leptin’s effects on energy metabolism are partly mediated by the hormone’s direct actions on peripheral tissues (6, 10, 22, 39). Alternative splicing of the L-R gene provides at least five unique L-R mRNA isoforms that encode proteins that differ in the length of their intracellular domains and that are expressed in multiple tissues at varying amounts (4, 44). The L-R isoform with a long intracellular domain is highly expressed in the hypothalamus and is believed to be primarily responsible for mediating leptin-induced signal transduction (13); however, short L-R isoforms might also transduce a leptin signal (17, 27). In skeletal muscle and other peripheral tissues, the majority of L-R mRNAs expressed encode for the short forms of the receptor (5, 10, 13, 44). The functions of the different L-R isoforms have not been established, but our data provide evidence that peripheral L-Rs mediate acute alterations in muscle lipid metabolism and suggest that the peripheral actions of leptin complement its central actions.

Similar effects of leptin on FA partitioning in isolated pancreatic islets were observed after chronic incubation with leptin (25). In isolated islets incubated for 48 h, leptin increases mRNA abundance for enzymes that promote FA oxidation: acyl-CoA oxidase, carnitine palmitoyltransferase, and uncoupling protein-2 (49). Furthermore, in these islets, leptin decreases mRNA abundance for enzymes involved in lipogenesis, such as acetyl-CoA carboxylase and mitochondrial glycerol-3-phosphate acyltransferase. In vivo leptin treatments increase mRNA expression of skeletal muscle uncoupling protein-3, a mitochondrial protein that is upregulated in response to starvation and exercise, physiological states in which muscle FA oxidation is enhanced (38, 45). These changes in mRNA abundance might explain why chronic, in vivo leptin treatments deplete the TAG content of adipose tissue, liver, pancreas, and...
skeletal muscle (25), but it is unclear whether the effects observed during our 90-min incubations could have resulted from alterations in gene expression.

Because leptin injections given to ob/ob mice dramatically improve glucose metabolism and insulin sensitivity, we hypothesized that leptin might directly increase insulin-stimulated glycogen synthesis in muscle. Instead, we found that leptin has no effect on glucose metabolism in muscles from lean animals (26), whereas in ob/ob muscles leptin decreased glycogen synthesis, similar to a previous report (23). Other in vitro studies have demonstrated that leptin has no direct effects on basal or insulin-stimulated glucose uptake in muscles isolated from either lean or ob/ob mice (50). Similarly, in rats, short-term in vivo leptin administration had no effect on muscle glycogen synthesis or 2-deoxyglucose uptake (34). Another report suggests that muscle responses to leptin may depend on previous leptin exposure. In C57BL/6J mice, leptin injections acutely (2 h) decreased whole body glucose clearance, but, after chronic (7 days) treatment, leptin increased insulin-stimulated muscle glycogen synthesis in vivo and increased soleus muscle glucose uptake in vitro (16). Taken together, these observations indicate that improvements in glucose homeostasis reported in leptin-treated ob/ob mice are mediated by long-term regulatory factors requiring chronic leptin exposure. Furthermore, leptin's effects on energy regulation occur largely through centrally mediated neuroendocrine responses that increase sympathetic output, thermogenesis, and energy expenditure and decrease food intake and body weight (1). Thus leptin-regulated adjustments in glucose homeostasis and insulin sensitivity might also require central factors that are absent from in vitro preparations.

The etiology of muscle insulin resistance is complex and still largely undefined. Muscle lipid accumulation appears to be linked to insulin resistance. For example, obesity and type II diabetes are strongly associated with increased muscle TAG and DAG content, and decreased FA oxidation (3, 7, 15, 28, 31, 42) and reversal of insulin resistance are associated with depletion of muscle lipid (28, 35). It has been hypothesized that perturbed muscle lipid metabolism, leading to TAG accumulation, contributes to the pathogenesis of insulin resistance (15, 31). The mechanism(s) by which lipid accumulation could interfere with glucose metabolism might involve alterations in lipid modulators of insulin-signaling pathways, such as DAG and acyl-CoA (32, 36). We found that, even though muscles from ob/ob mice were insulin resistant, when insulin was added to the media, ob/ob muscles preferentially partitioned FA toward TAG and away from β-oxidation, thereby increasing the ratio of TAG partitioned into TAG/CO₂ by more than twofold compared with basal muscles. Data from this study and our earlier report (26) are the first to show that insulin suppression of FA oxidation is directly associated with a concomitant increase in the amount of FA incorporated into neutral lipid and that insulin regulates muscle FA partitioning, even in obese, insulin-resistant states. In human studies, hyperglycemic, hyperinsulinemic clamping markedly reduces whole body FA oxidation, but effects on muscle TAG have not been reported. Although the concentrations of hormones used in this study were 10- to 100-fold higher than in serum, others have demonstrated that these relative doses are required to elicit maximal hormone responses in incubated skeletal muscle (12, 18, 26). Supraphysiological hormone concentrations may be required because the muscle fiber architecture does not permit optimal exposure of receptors to hormones.

Insulin's effect on muscle FA partitioning appears to be mediated by activation of PI 3-kinase, since it was completely blocked by wortmannin. Wortmannin also blocks insulin-stimulated glucose uptake (21, 46), suggesting that insulin's effects on FA partitioning might be secondary to insulin-stimulated glucose uptake. Alternatively, insulin's regulation of muscle lipid metabolism could be due to PI 3-kinase-mediated inactivation of oxidative enzymes and/or activation of lipogenic
enzymes. Our observation that increasing the media glucose concentration decreased muscle FA oxidation suggests that acute insulin suppression of muscle FA oxidation is at least partly due to increased glucose uptake. Increased glucose uptake results in increased muscle concentrations of citrate, which allosterically activates acetyl-CoA carboxylase and stimulates the production of malonyl-CoA (37). Malonyl-CoA, a key inhibitor of FA oxidation, is thus the likely link between insulin-stimulated glucose uptake and insulin inhibition of FA oxidation. In contrast, insulin's stimulatory effect on FA esterification in muscle lipids is likely mediated by acute activation of one or more of the enzymes involved in glycerolipid biosynthesis, since increasing the media glucose concentration in the absence of insulin did not augment TAG synthesis. Furthermore, these data suggest that the low rates of FA oxidation and the increased concentrations of TAG previously reported in muscle from obese animals (3, 35) and humans (7, 15) were probably due in part to chronic hyperinsulinemia. Because we found that leptin attenuated insulin's antioxidative and lipogenic actions, leptin's ability to improve insulin sensitivity in vivo might be partly mediated by its inhibition or reversal of muscle TAG accumulation (40).

It has been suggested that leptin and insulin are counterregulatory hormones that regulate energy homeostasis (33). In isolated rat adipocytes, leptin diminishes insulin-stimulated glucose uptake and attenuates insulin's antilipolytic and lipogenic effects (25). In isolated pancreatic islets and in perfused pancreas, leptin inhibits insulin secretion, and, in Hep G2 hepatocytes, leptin inhibits insulin-induced decreases in phosphoenolpyruvate carboxykinase expression (6). We found that, in isolated muscle, leptin opposes the antioxidative and lipogenic effects of insulin. The ability of leptin to attenuate insulin's actions on peripheral glucose and lipid metabolism is consistent with observations that leptin modulates the phosphorylation of insulin-signaling proteins. In Hep G2 hepatocytes, leptin downregulates insulin-dependent phosphorylation of the insulin receptor substrate (IRS)-1 (6). Similarly, in Rat1 fibroblasts, leptin impairs phosphorylation of the insulin receptor and of IRS-1 (19). Leptin may not, however, oppose insulin action in every tissue. For example, in cultured C2C12 myotubes, which express only the short L-R, leptin mimicked insulin's actions by stimulating glucose uptake and glycogen synthesis through a PI 3-kinase-dependent pathway (2, 17). Likewise, in Hep G2 cells, leptin increases PI 3-kinase activity and its association with IRS-1, and in pancreatic islets leptin-induced activation of phosphodiesterase 3B is also mediated by PI 3-kinase (48). These observations, together with our data showing that wortmannin attenuated leptin's effects on muscle lipid partitioning, suggest that activation of PI 3-kinase might be common to both the leptin and insulin-signaling cascades. These observations also imply that, in some peripheral tissues, leptin and insulin-signaling pathways may converge, and, furthermore, that the interactions between leptin and insulin are tissue specific and may be related to the expression pattern of distinct L-R isoforms.

Muscles from ob/ob mice are likely to contain more intermuscular fat than muscle from lean animals. Because any adipose tissue present would contribute minimally to total oxidation, the presence of intermuscular fat should not affect FA oxidation. If hormonal effects on TAG synthesis were due largely to contaminating adipose tissue, one would expect that the effects would be greater for ob/ob muscle (which contains more intermuscular fat). Instead, our data showed effects on TAG synthesis that were similar or greater in lean compared with ob/ob muscle, suggesting that the results were due to the regulation of lipogenesis in muscle, rather than adipose tissue.

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