Insulin increases FA uptake and esterification but reduces lipid utilization in isolated contracting muscle

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A recent study (38) has demonstrated that insulin-induced inhibition of FA oxidation has also been observed in isolated rodent muscle (10, 31). Hence, it is considered evidence to support the idea that insulin and/or glucose can inhibit FA oxidation in resting skeletal muscle.

It is well known that insulin increases the activities of lipogenic enzymes in a number of tissues, although these responses are tissue specific (6). It was only recently reported that insulin can increase FA esterification in resting skeletal muscle (30, 31) while simultaneously lowering the rate of FA oxidation in this tissue (1, 30, 31). Interestingly, it appeared that insulin increased the net uptake of FA [i.e., sum of FA triacylglycerol (TG) esterification plus FA oxidation] (30, 31). However, the effects of insulin on the esterification of FA into other endogenous lipid pools [e.g., phospholipid (PL), diacylglycerol (DG)] or on TG hydrolysis have not been examined. Nevertheless, these data (30, 31) raise the interesting specter that, in muscle, insulin not only promotes glycogen storage, but also TG storage. Whether insulin inhibits TG hydrolysis in this tissue has not been directly examined.

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"Reverse" glucose-FA cycle. When glucose availability is enhanced, FA oxidation in muscle is suppressed (38), an effect that may be mediated by an increase in malonyl-CoA content and subsequent inhibition of carnitine palmitoyltransferase I (12). Hyperinsulinemia, whether during euglycemic (20) or hyperglycemic conditions (38), also inhibits FA oxidation. Insulin-induced inhibition of FA oxidation has also been observed in isolated rodent muscle (1, 30, 31). Thus there is considerable evidence to support the idea that insulin and/or glucose can inhibit FA oxidation in resting skeletal muscle.

It is well known that insulin increases the activities of lipogenic enzymes in a number of tissues, although these responses are tissue specific (6). It was only recently reported that insulin can increase FA esterification in resting skeletal muscle (30, 31) while simultaneously lowering the rate of FA oxidation in this tissue (1, 30, 31). Interestingly, it appeared that insulin increased the net uptake of FA [i.e., sum of FA triacylglycerol (TG) esterification plus FA oxidation] (30, 31). However, the effects of insulin on the esterification of FA into other endogenous lipid pools [e.g., phospholipid (PL), diacylglycerol (DG)] or on TG hydrolysis have not been examined. Nevertheless, these data (30, 31) raise the interesting specter that, in muscle, insulin not only promotes glycogen storage, but also TG storage. Whether insulin inhibits TG hydrolysis in this tissue has not been directly examined.
Muscle contraction also increases the uptake of FA, but this is largely attributable to an increased rate of FA oxidation. Interestingly, just as with insulin, the absolute rate of TG synthesis is also increased during muscle contraction. This increase in TG synthesis is quite similar in contracting (+60%) (8) and insulin-stimulated muscle (+30%) (30, 31), although a much greater increase in the rate of TG lipolysis and oxidation during muscle contraction results in a net reduction in the TG depots (8). Thus contraction and insulin increase FA uptake by muscle, while the effects of these physiological stimuli on FA oxidation differ, and the effects on TG synthesis appear to be similar. Whether the effects of insulin and contraction on TG synthesis in muscle are additive remains to be determined. Furthermore, it is not known whether insulin also inhibits FA oxidation in contracting muscle, as has been observed during rest.

It is generally acknowledged that circulating insulin levels rapidly decline to low levels at the onset of exercise (45). However, despite a reduction in circulating levels, insulin still appears to have important metabolic effects during exercise, as indicated by lower muscle glucose uptake in insulin-deficient humans (43). We speculate that the effect of insulin on muscle FA metabolism during contraction may be of considerable significance. Specifically, a decline in circulating insulin levels may be necessary to fully increase FA oxidation in contracting skeletal muscle. Therefore, the purpose of this study was to examine the effects of insulin on FA metabolism in resting and contracting isolated rat soleus muscle. The pulse-chase technique (8, 9, 34) was used to simultaneously assess FA oxidation and esterification and TG hydrolysis. We hypothesized that, at rest, insulin enhances the uptake of FA into muscle, resulting in increased rates of PL, DG, and TG esterification, and decreases the rates of FA oxidation and TG hydrolysis. We also hypothesized that insulin and contraction have an additive effect on FA uptake and esterification and that insulin inhibits FA oxidation during muscle contraction. Finally, it has previously been demonstrated that insulin’s stimulation of TG esterification and inhibition of oxidation at rest are reduced when phophatidylinositol 3-kinase (PI 3-kinase) is inhibited (30). Thus, in the present study, we also determined whether insulin’s modulatory effects on FA metabolism during contraction would be reduced in the presence of the PI 3-kinase inhibitor LY-29004.

METHODS

Animals

Fifty female Sprague-Dawley rats (195 ± 7 g) were used in these experiments. Twenty-five animals were used in each of the resting and contraction conditions [with and without insulin (n = 15) and with and without PI 3-kinase inhibitor (n = 10)]. Animals were housed in a controlled environment on a reversed 12:12-h light-dark cycle and fed Purina rat chow ad libitum. Ethical approval was obtained from the Animal Ethics Committees at the Universities of Waterloo and Guelph. Animals were anesthetized with pentobarbital sodium (6 mg/100 g body mass ip) before any experimental procedures.

Preparation of Soleus Muscle Strips

Longitudinal strips of soleus [60% type I, 31% type IIA (9)] were carefully dissected without damage, tied at the tendons, mounted between two brass hooks, and placed in a 7-ml glass incubation reservoir containing 7 ml of warmed (30°C) Krebs-Henseleit buffer gassed with 95% O2-5% CO2 (pH 7.4) containing 4% BSA (FA free; Roche Diagnostics, Laval, PQ, Canada), 5 mM glucose, and 1.0 mM palmitate. Palmitic acid was dissolved in ethanol, and a small volume (0.8% of the total buffer volume) was added to the incubation buffer to achieve the final desired palmitate concentration. No ethanol was detected in the buffer after gassing was completed.

Pulse-Chase Procedures

Pulse. Muscles were incubated for 30 min at 30°C with 1 mM palmitate and 3 μCi/vial of [3H]palmitate (Amersham Life Science, Oakville, ON, Canada) to “load” the endogenous lipid pools (monoacylglycerol, DG, TG, and PL) with [3H]palmitate.

Wash. Muscles were then washed for 20 min by placing them in new vials containing gassed buffer with 1.0 mM palmitate, but no radiolabeled palmitate. This was required to remove [3H]palmitate trapped in the interstitium, which would represent a “contaminant” source of labeled palmitate during the subsequent chase phase.

Chase. After the wash, some muscles were removed and processed for lipid extraction, while other muscles were incubated for an additional period for the chase experiments. In these experiments, muscles were incubated (chased) in the presence or absence of a maximal (i.e., pharmacological) insulin dosage (10 mU/ml) for an additional 60 min at rest or while electrically stimulated to contract (30 min). Muscles were stimulated with 150-ms trains of 0.1-ms impulses (20–40 V, 60 Hz) at 20 tetani/min. This was previously determined to elicit maximal rates of TG pool turnover and oxidation without any significant development of fatigue (8, 9, 34). Muscles were chased in buffer containing 1.0 mM palmitate with 2 μCi of [1-14C]palmitate (Amersham Life Science). This permitted monitoring of exogenous palmitate oxidation and incorporation into endogenous lipids during simultaneous determination of changes in the 3H content in the same endogenous pools into which [3H]palmitate had been previously incorporated during the pulse. In this manner, it was possible to determine concomitantly the contributions of the endogenous lipids (i.e., changes in 3H label in lipid pools and 3H2O production) and the exogenous FA-to-muscle metabolism (i.e., [14C]palmitate deposition into lipid pools and oxidation measured by 14CO2 production).

In a separate set of incubations, soleus strips were incubated at rest and during contraction with a maximal dosage of insulin in the presence or absence of the PI 3-kinase-specific inhibitor LY-294002 (50 μM). This concentration has previously been demonstrated to inhibit insulin-stimulated glucose uptake (44).

Extraction of Muscle Lipids

Muscles were placed in 13-ml plastic centrifuge tubes containing 5.0 ml of ice-cold 1:1 chloroform-methanol (vol/vol) and homogenized 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 described above to separate the aqueous and lipophilic phases. One milliliter of the aqueous phase was quantified by liquid scintillation counting to determine the amount of $^{14}$C-labeled oxidative intermediates resulting from isotopic exchange. The quantification of labeled oxidative intermediates was used to correct measurements of exogenous FA oxidation (i.e., $^{14}$CO$_2$ production). This correction factor was about twofold and is similar to that reported previously (8).

The chloroform phase, containing the total lipids extracted from muscle, was gently evaporated under a stream of N$_2$. Lipids were redissolved in 2.1 chloroform-methanol containing ~5 mg of lipids (TG, DG, and PL; Sigma Chemical, St. Louis, MO) to identify the lipid bands on the silica gel plates. Samples were spotted onto oven-dried silica gel plates (Fisher Scientific, Mississauga, ON, Canada) and placed in a sealed tank containing solvent (60:40:3 heptane-isopropyl ether-acetic acid) for 40 min. Plates were air-dried, sprayed with dichlorofluorescein dye (0.02% wt/vol in ethanol), and visualized under long-wave ultraviolet light. Individual lipid bands were marked on the plate with a scalpel and scraped into vials for liquid scintillation counting.

**Measurement of Endogenous and Exogenous Oxidation**

Gaseous $^{14}$CO$_2$ produced from the exogenous oxidation of [1-$^{14}$C]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 H$_2$SO$_4$ and 0.5-ml microcentrifuge tube containing 1 M benzenethionium hydroxide. Liberated $^{14}$CO$_2$ was trapped in the benzenethionium hydroxide over 60 min, and the microcentrifuge tube containing the trapped $^{14}$CO$_2$ was placed in a scintillation vial and counted. In a separate experiment, complete recovery of $[^{14}$C]bicarbonate (98.3 ± 3.0%) was confirmed.

**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 disintegrations per minute per total palmitate in nmol). Hydrolysis of intramuscular lipids was calculated from the loss of pre-loaded $[^{3}$H]palmitate from each pool.

Results were analyzed using analysis of variance procedures, and a Student's $t$-test was used to test significant differences between insulin and insulin-free conditions revealed by the analysis of variance. Significance was accepted at $P \leq 0.05$. Values are means ± SE.

**RESULTS**

**Effects of Insulin on FA Metabolism in Resting, Isolated Soleus Muscle**

At rest, insulin significantly increased the total palmitate uptake (PL, DG, and TG esterification plus oxidation) into soleus muscle (+49%, $P < 0.05$; Fig. 1). The rate of PL formation showed a trend toward enhancement with insulin (Table 1), but this was not significant ($P > 0.05$), whereas the rates of intramuscular DG (+37%, $P < 0.05$; Table 1) and TG synthesis (+61%, $P < 0.05$; Fig. 2A) were significantly increased by insulin. In contrast, insulin reduced the rate of palmitate oxidation by 38% (Fig. 3; $P < 0.05$), resulting in an almost threefold increase in the amount of palmitate partitioned toward TG esterification relative to oxidation (Fig. 4). The hydrolysis of TG was significantly blunted with insulin ($P < 0.05$; Fig. 2B), but PL and DG pools were unaffected by insulin in resting muscle (Table 1).

**Effects of Contraction on FA Metabolism in Isolated Soleus Muscle**

During muscle contraction, the total uptake of palmitate was greatly increased by +421% ($P < 0.001$; Fig. 1). This was accompanied by increased rates of FA esterification into PL (+283%, $P < 0.01$; Table 1), DG (+51%, $P < 0.05$; Table 1), and TG (+61%, $P < 0.05$; Fig. 2A) pools and a simultaneous increase in the loss of $[^{3}$H]palmitate from TG (+140%, $P < 0.01$; Fig. 2B). FA oxidation increased ~20-fold ($P < 0.001$; Fig. 3A).

During contraction, there was a relative partitioning of FA away from TG esterification and toward oxidation (resting esterification-to-oxidation ratio = 5.8 ± 0.8, contracting esterification-to-oxidation ratio = 0.4 ± 0.1, $P < 0.001$; Fig. 4). This was a result of the much greater increase in FA oxidation relative to the small increase in esterification.

**Effects of Insulin on FA Metabolism in Contracting, Isolated Soleus Muscle**

The stimulatory effect of insulin on FA uptake and esterification was additive to that of contraction. In the presence of insulin, total palmitate uptake was further increased in contracting soleus muscle (+21%, $P < 0.05$; Fig. 1). This resulted in enhanced rates of FA esterification into PL (+73%, $P < 0.05$; Table 1), DG (+19%, $P < 0.05$; Table 1), and TG (+161%, $P < 0.01$; Fig. 2A) relative to that of contraction alone. Thus the amount of palmitate taken up and esterified in con-
tracting muscle in the presence of insulin was similar to the sum of palmitate incorporated and esterified under the independent conditions of insulin and contraction (Fig. 5). In contrast, the rates of palmitate oxidation (224%, P < 0.05; Fig. 3) and TG hydrolysis (283%, P < 0.01; Fig. 2B) were significantly blunted during contraction by insulin. Thus the effect of insulin on FA partitioning during contraction was remarkably similar to that observed at rest, i.e., about a threefold increase in the relative partitioning toward esterification and away from oxidation (Fig. 4).

Effects of the PI 3-Kinase Inhibitor LY-294002 on Muscle FA Metabolism

Overall, during rest and contraction, the PI-3-kinase inhibitor LY-294002 reduced insulin’s stimulatory effect on total palmitate uptake and esterification to PL and TG and inhibition of TG hydrolysis (P < 0.05; data not shown). Specifically, during muscle contraction, the modulatory effects of insulin on FA metabolism were blunted in the presence of LY-294002 (Fig. 6). In the presence of LY-294002, FA oxidation (+48%, P < 0.05) and TG hydrolysis (+100%, P < 0.01) were re-

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Table 1. Esterification and hydrolysis of PL and DG pools

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<thead>
<tr>
<th>Esterification</th>
<th>Hydrolysis</th>
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<td>PL</td>
<td>DG</td>
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<tr>
<td>PL</td>
<td>DG</td>
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<tr>
<td>Rest</td>
<td></td>
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<tr>
<td>-Insulin</td>
<td>16.2 ± 1.4</td>
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<tr>
<td>+Insulin</td>
<td>19.7 ± 3.9</td>
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<td></td>
<td>-0.4 ± 6.5</td>
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<td></td>
<td>-2.7 ± 3.2</td>
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<tr>
<td>Contraction</td>
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<tr>
<td>-Insulin</td>
<td>62.1 ± 9.9†</td>
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<tr>
<td>+Insulin</td>
<td>107.7 ± 4.8†</td>
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<tr>
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<td>0.1 ± 2.1</td>
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Values are means ± SE expressed in nmol palmitate·g wet wt⁻¹·60 min⁻¹; n = 15/group. PL, phospholipid; DG, diacylglycerol. *Significantly different from insulin-free condition. †Significantly different from rest.

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Fig. 2. Triacylglycerol esterification (A) and hydrolysis (B) in soleus in the presence and absence of insulin (10 mU/ml). a Significantly different from insulin-free condition; b significantly different from rest; n = 15 per group.

Fig. 3. Exogenous palmitate oxidation in soleus in the presence and absence of insulin (10 mU/ml). a Significantly different from insulin-free condition; b significantly different from rest; n = 15 per group.

Fig. 4. Ratio of total lipid esterification to oxidation in soleus in the presence and absence of insulin (10 mU/ml). a Significantly different from insulin-free condition; b significantly different from rest; n = 15 per group.
stored, whereas insulin’s stimulation of total FA uptake (−24%, \( P < 0.05 \)) and esterification to PL, DG, and TG (−33%, \( P < 0.05 \)) were reduced.

DISCUSSION

We have examined the effects of insulin on FA metabolism and TG hydrolysis in isolated muscles at rest and during muscle contraction. In this study, we utilized the pulse-chase technique to examine the acute effect of insulin on muscle FA metabolism and TG hydrolysis in isolated soleus muscle. We previously used this pulse-chase procedure to compare differences in lipid metabolism in metabolically heterogeneous muscles (9) and to compare the effects of epinephrine (34) and muscle contraction (8) on skeletal muscle lipid metabolism. This isolated muscle preparation allows for the examination of the direct effects of insulin on muscle lipid metabolism in the absence of other systemic perturbations (e.g., altered lipid and CHO supply, increased catecholamines). However, the findings derived using this model (isolated muscle, pharmacological hormonal concentrations) are difficult to apply to an in vivo situation and warrant further investigation. Furthermore, because lipid pools are measured in the whole muscle, it cannot be excluded that a source of lipids are adipocytes between the muscle bundles. However, given the pronounced stimulation of FA metabolism that we observe during contraction (which would not be expected to alter adipocyte FA metabolism in the absence of hormonal changes or an intact neural supply), it is most likely that the perturbations in metabolism observed in our experiments are a direct reflection of changes in the myocyte. It has also been previously demonstrated that interfiber adipocyte contamination is negligible in soleus muscles from lean rats (25).

Effect of Insulin on Basal Muscle Lipid Metabolism

According to the classical glucose-FA cycle theory, impaired insulin sensitivity in skeletal muscle might be explained by the preferential oxidation of FA, resulting in the subsequent inhibition of pyruvate dehydrogenase and phosphofructokinase by the accumulation of acetyl-CoA and citrate, respectively. However, the fact that acute lipid infusion decreases insulin sensitivity several hours after the initial decrease in glucose oxidation (4) directly challenges the validity of the glucose-FA cycle as the mechanism by which lipids alter insulin sensitivity. Furthermore, recent evidence suggests that FA oxidation in skeletal muscle from obese/insulin-resistant individuals is actually decreased in the postabsorptive condition (21, 39), whereas intramuscular TG content is also increased in these individuals (23, 33). This indicates that a repartitioning of FA metabolism (i.e., toward esterification and away from oxidation) is a key feature of altered skeletal muscle metabolism in obesity.

Fig. 5. Additive effect of insulin and contraction on total fatty acid uptake (A) and total esterification of fatty acids into phospholipids, diacylglycerol, and triacylglycerol (B); \( n = 15 \) per group.

Fig. 6. Insulin’s effects on muscle lipid metabolism are reversed by the phosphatidylinositol 3-kinase inhibitor LY-294002. *Significantly different from condition without LY-294002. Insulin was present in all incubations; \( n = 10 \) per group.
The findings of the present study support the hypothesis that hyperinsulinemia leads to depressed FA oxidation and enhanced storage in skeletal muscle. These findings confirm very recent reports in isolated rodent preparations (1, 30, 31). These effects of insulin on skeletal muscle FA metabolism parallel the FA repartitioning in skeletal muscle observed in hyperinsulinemic, obese, insulin-resistant patients (19, 21, 23). This is also in agreement with a recent study that has clearly demonstrated that FA oxidation is reduced in homogenates prepared from muscles obtained from obese individuals (22). It is tempting, therefore, to suggest that hyperinsulinemia promotes the repartitioning of FA metabolism in skeletal muscle. However, it is unknown whether the hyperinsulinemic condition prevalent in obese, insulin-resistant individuals is responsible for the repartitioning of FA away from oxidation and toward storage. In a recent study, the infusion of insulin to supraphysiological concentrations suppressed whole body lipid oxidation in lean, but not in obese, individuals (19). However, the basal rate of lipid oxidation was already approximately 50% lower in the obese than in the lean individuals and was associated with a nearly twofold greater circulating insulin concentration. Thus the failure to further decrease lipid oxidation in obesity does not eliminate the possibility that the already elevated basal concentrations of insulin were responsible for the depressed rate of fat oxidation under postabsorptive conditions.

Depressed glycerol release from muscle, as an index of reduced intramuscular lipolysis, has previously been demonstrated during a hyperinsulinemic clamp (13, 18, 26). However, we are unaware of any reports directly measuring changes in muscle lipid (PL, DG, TG) turnover in response to insulin. We present the first direct evidence that insulin impairs intramuscular TG hydrolysis. Our data indicate that hyperinsulinemia would be expected to result in the accumulation of intramuscular DG and TG as a result of the stimulatory effects of insulin on FA esterification and the blunting of TG hydrolysis. The accumulation of intramuscular TG is associated with insulin resistance (23, 33), and an increase in intracellular DG may activate various isoforms of protein kinase C, particularly PKC-θ (2), which inhibits tyrosine kinase activity of the insulin receptor and insulin receptor substrate 1 (29, 37).

In the present study, the stimulatory effects of insulin on total FA uptake (+41%) and esterification of PL (+22%), DG (+60%), and TG (+61%) were similar, suggesting that the enhanced rates of esterification may be the result of an enhanced transport of FA into the cell. We now have preliminary evidence that insulin induces the translocation of FAT/CD36 to the sarcolemma (unpublished findings).

**Effect of Insulin on Muscle Lipid Metabolism During Contraction**

Acute muscular contraction has been demonstrated to increase hormone-sensitive lipase (HSL) activity in mouse soleus (24). In agreement with this finding, muscle contraction, independent of hormonal influence, increases intramuscular TG utilization (8, 17). The mechanism underlying the contraction-induced increase in HSL activity is unknown. Furthermore, whether intramuscular HSL activity can be hormonally regulated during muscular contraction is also unclear. To our knowledge, no studies have examined the effect of hormones, such as insulin or epinephrine, on intramuscular TG utilization during exercise. It has been recently argued that the regulation of HSL in skeletal muscle by external hormonal signals during exercise is unlikely, inasmuch as Ca^{2+} and metabolites related to the energy status of the cell are expected to be the dominant regulators (40). However, this may not be the case, since a surprising finding of this study was that insulin plays a pivotal role in the regulation of FA oxidation and TG utilization during exercise. It suggests that well-known early decline in insulin during exercise may be a major factor in permitting increased lipid utilization by muscle. The role of epinephrine in regulating intramuscular lipolysis during contraction is also relatively unexamined. Infusion of various physiological concentrations of epinephrine during low-intensity cycling (25% peak O_2 uptake) enhances whole body lipolysis but actually reduces whole body lipid oxidation (28). Furthermore, increasing the exercise intensity to 45% peak O_2 uptake blunts the lipolytic action of epinephrine (28). Thus the most important hormonal signal early in exercise that permits increased lipid utilization by muscle may be the decline in insulin, rather than the increase in epinephrine. The importance of each of these hormones in regulating muscle FA metabolism during exercise clearly requires further study.

The findings of the present study also clearly demonstrate that the stimulatory effects of insulin and contraction on FA uptake and lipid esterification are additive (Fig. 5). The fact that this additivity is observed in all the major lipid pools (PL, DG, TG) suggests that the enhanced rates of esterification may be secondary to altered FA transport into the cell. It has recently been demonstrated that one of the FA transporters, FAT/CD36, can be translocated from an intracellular pool to the plasma membrane (5). Thus the data of the present study suggest the interesting possibility that there may be contraction and insulin-sensitive pools of FAT/CD36, analogous to that observed for the GLUT-4 transporter.

**Mechanisms Underlying Insulin’s Modulatory Effects on Muscle FA Metabolism**

The mechanisms underlying insulin’s potential regulation of muscle lipid metabolism have remained relatively unexamined. A recent study by Muoio et al. (31) demonstrated that insulin’s repartitioning of FA toward esterification and away from oxidation was eliminated in the presence of the PI 3-kinase inhibitor wortmannin. The results of the present study are in
agreement with these findings and, importantly, extend these findings by implicating a role for PI 3-kinase in regulating muscle FA metabolism during intense contraction. It has been suggested that PI 3-kinase may inactivate oxidative enzymes and/or activate lipogenic enzymes (31). Alternatively, Muoio et al. also suggested that stimulation of glucose uptake by PI 3-kinase will lead to the enhanced production of malonyl-CoA and subsequent inhibition of FA oxidation. Insulin’s “anti-oxidative/prolipogenic” effects in skeletal muscle may also be regulated by AMP-dependent protein kinase (AMPK). Stimulation of AMPK with imidazolecarboxamide riboside (AICAR) in rodent muscles results in the inhibition of acetyl-CoA carboxylase and a subsequent decrease in muscle malonyl-CoA, resulting in enhanced lipid oxidation (27) and a repartitioning of FA toward oxidation and away from esterification (32). Furthermore, insulin’s effects on lipid partitioning (i.e., enhanced esterification, decreased oxidation) are eliminated in the presence of AICAR, suggesting that insulin may alter muscle lipid metabolism by decreasing AMPK activity (32). However, the possibility that AICAR eliminates insulin’s effects on the repartitioning of FA during contraction was not examined in the present study.

Although HSL has been detected in skeletal muscle (16, 24), the enzymatic regulation of TG hydrolysis in this tissue is very poorly understood. Muscle HSL activity can be stimulated with epinephrine and cAMP-dependent protein kinase and eliminated by the β-adrenergic agonist propanolol (24). However, we are unaware of any direct evidence that demonstrates insulin’s ability to regulate HSL in skeletal muscle. Thus the findings of the present study are important, in that they establish that 1) insulin has a potent inhibitory effect on TG lipolysis at rest and during contraction and 2) these effects are mediated at least in part by the activation of PI 3-kinase, since insulin’s inhibition of lipolysis can be reversed in the presence of the inhibitor LY-294002.

Summary

The present study has demonstrated that insulin has potent antilipolytic and antioxidative effects, as well as a prolipogenic effect in resting skeletal muscle. This may have important ramifications regarding the accumulation of intramuscular lipids in insulin-resistant individuals. Importantly, these effects are maintained in the face of dramatically increased energy requirements (i.e., contraction), suggesting that the decline in insulin may play a pivotal role in increasing the use of lipids by skeletal muscle during exercise. Insulin’s effects on muscle lipid metabolism during contraction are reversed in the presence of LY-294002, indicating the importance of PI 3-kinase mechanistically. Finally, the increased esterification of the intracellular lipid pools in insulin- and contraction-stimulated conditions is related to increases in the total FA uptake, suggesting the presence of two intracellular pools of the putative FA transporter FAT/CD36. Although it must be acknowledged that the results of this study cannot be directly applied to the in vivo situation, our findings clearly indicate that insulin is capable of modulating lipid utilization in muscle at rest and during contraction and is a potential cause of the depressed rates of lipid oxidation and enhanced TG storage observed in the hyperinsulinemic, obese condition.

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