Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-α

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Submitted 1 April 2004; accepted in final form 14 May 2004

Bruce, Clinton R., and David J. Dyck. Cytokine regulation of skeletal muscle fatty acid metabolism: effect of interleukin-6 and tumor necrosis factor-α. Am J Physiol Endocrinol Metab 287: E616–E621, 2004.—IL-6 and TNF-α have been associated with insulin resistance and type 2 diabetes. Furthermore, abnormalities in muscle fatty acid (FA) metabolism are strongly associated with the development of insulin resistance. However, few studies have directly examined the effects of either IL-6 or TNF-α on skeletal muscle FA metabolism. Here, we used a pulse-chase technique to determine the effect of IL-6 (50–5,000 pg/ml) and TNF-α (50–5,000 pg/ml) on FA metabolism in isolated rat soleus muscle. IL-6 (5,000 pg/ml) increased exogenous and endogenous FA oxidation by ~50% (P < 0.05) but had no effect on FA uptake or incorporation of FA into endogenous lipid pools. In contrast, TNF-α had no effect on FA oxidation but increased FA incorporation into diacylglycerol (DAG) by 45% (P < 0.05). When both IL-6 (5,000 pg/ml) and insulin (10 mM) were present, IL-6 attenuated insulin’s suppressive effect on FA oxidation, increasing exogenous FA oxidation (+37%, P < 0.05). Furthermore, in the presence of insulin, IL-6 reduced the esterification of FA to triacylglycerol by 22% (P < 0.05). When added in combination with IL-6 or leptin (10 µg/ml), the TNF-α-induced increase in DAG synthesis was inhibited. In conclusion, the results demonstrate that IL-6 plays an important role in regulating fat metabolism in muscle, increasing rates of FA oxidation, and attenuating insulin’s lipogenic effects. In contrast, TNF-α had no effect on FA oxidation but increased FA incorporation into DAG, which may be involved in the development of TNF-α-induced insulin resistance in skeletal muscle.

In addition to adiponectin and leptin, adipocytes secrete a number of other proteins that may influence metabolism and insulin action, such as the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Insulin resistance has been associated with elevated levels of plasma TNF-α (41), and its expression is increased in adipose tissue (11) and skeletal muscle of insulin-resistant humans (25). Moreover, long-term exposure to TNF-α induces insulin resistance in rodents (18), whereas neutralization of TNF-α improves insulin action in obese rats (12). TNF-α may induce insulin resistance by inhibiting the activation of insulin receptor substrate (IRS)-1 (13) and by elevating plasma free fatty acids via stimulation of adipose tissue lipolysis (9). Furthermore, in cell culture, TNF-α stimulates the production of diacylglycerol (DAG) and ceramide (29), which have been implicated in the pathogenesis of skeletal muscle insulin resistance (1, 39).

IL-6 is a biologically active substance that is not only secreted by adipocytes but is also released from contracting skeletal muscle (31). Interestingly, elevated levels of plasma IL-6 have been associated with the development of insulin resistance (3); however, there is little evidence to suggest that IL-6 causes insulin resistance in vivo. In fact, it is difficult to believe that IL-6 would impair glucose uptake, given that it is upregulated in contracting muscle when the requirement for glucose uptake is augmented (30). Indeed, acute administration of IL-6 did not impair glucose homeostasis in healthy humans (30). Furthermore, IL-6 infusion stimulated lipolysis and rates of whole body fat oxidation in humans (36), suggesting that IL-6 is a regulator of FA metabolism.

To date, few studies have examined the direct effect of either TNF-α or IL-6 on skeletal muscle metabolism and, in particular, FA metabolism. Because skeletal muscle is the major tissue contributing to basal metabolic rate and is the primary tissue responsible for whole body glucose and FA metabolism (42), studies examining the effect of these cytokines on muscle metabolism are warranted. Hence, the aim of the present study was to examine the effect of TNF-α and IL-6 on skeletal muscle FA metabolism. In the present study, we applied a pulse-chase technique to simultaneously assess endogenous FA oxidation as well as esterification and oxidation of exogenous FA in isolated rat soleus muscle. We hypothesized that IL-6 would stimulate FA oxidation and reduce palmitate incorporation into triacylglycerol, whereas TNF-α would decrease FA oxidation and increase FA incorporation into endogenous lipid pools.

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METHODS

Animals and Muscle Preparation

Female Sprague-Dawley rats weighing 195 ± 2 g (mean ± SE) were used for all experiments. Animals were housed in a controlled environment on a 12:12-h reversed light-dark cycle so that they would be awake during the day when experiments were performed, and they were fed Purina rat chow ad libitum. All procedures were approved by the University of Guelph Animal Care Committee. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body wt), and the soleus muscle was carefully dissected into longitudinal strips from tendon to tendon by use of a 27-gauge needle. Three strips were utilized from each soleus muscle, and the average strip mass was 25.1 ± 0.1 mg wet mass.

Strips were removed and placed in a 20-ml glass reaction vial containing 2.5 ml of warmed (30°C), pregassed (95% O2-5% CO2, pH 7.4), modified Krebs-Henseleit buffer containing 4% FA-free BSA (Roche, Indianapolis, IN), 10 mM glucose, and 1 mM palmitate. This was the base buffer used in all experiments. Dose-response experiments were conducted to examine the effect of cytokines (IL-6 and TNF-α) on FA metabolism. A leptin condition was also included, which served as a positive control.

Pulse-Chase Experiments

Preincubation and preexperimental labeling of the intramuscular lipid pools. Soleus muscle strips were incubated in 2.5 ml of base buffer for 30 min to permit equilibration. The pulse-chase procedures used have been described previously (7). Briefly, muscle strips were removed from the preincubation buffer and transferred to a second vial containing a pulse buffer consisting of the base buffer plus 2 μCi of [9,10-3H]palmitate (Amersham Life Sciences, Oakville, ON, Canada). Muscles were pulsed with [9,10-3H]palmitate for 40 min to prelabel endogenous lipid pools (triacylglycerol (TAG) and DAG). The muscle strip was transferred to a third vial with incubation medium containing no radiolabeled palmitate to wash nonincorporated [9,10-3H]palmitate (20 min).

Experimental phase (chase). After the wash, muscle strips were transferred to vials containing 0.5 μCi/ml [1-14C]palmitate (Amersham Life Sciences). In these experiments, muscles were chased in the presence of one of recombinant rat IL-6 (50, 500, or 5,000 pg/ml; Peprotech, Ottawa, ON, Canada), recombinant rat TNF-α (50, 500, or 5,000 pg/ml; Peprotech), or a maximal leptin dosage (10 μg/ml). The pulse-chase procedures used have been described previously (7). The muscle strips were incubated for 60 min to prelabel endogenous lipid pools (triacylglycerol (TAG) and DAG). The muscle strip was transferred to a third vial with incubation medium containing no radiolabeled palmitate to wash nonincorporated [9,10-3H]palmitate (20 min).

A

B

Fig. 1. Exogenous (A) and endogenous (B) palmitate oxidation in isolated soleus muscle. Soleus muscle strips were incubated with leptin (10 μg/ml), IL-6 (50, 500, or 5,000 pg/ml) or TNF-α (50, 500, or 5,000 pg/ml). CON, control. Values are means ± SE. All values are expressed as nmol/g wet wt; n = 8–10 per group. *Significantly different from CON, P < 0.05.
Measurement of endogenous and exogenous oxidation. \(^{3}\)H\(_2\)O produced from the endogenous oxidation of [9,10-\(^{3}\)H]palmitate was separated from the labeled substrate by transferring 1.0 ml of the chase incubation medium to a plastic centrifuge tube containing 5.0 ml of 2:1 chloroform-methanol (vol/vol). Samples were shaken for 10 min before addition of 2.0 ml of 2 M KCl-HCl, were shaken again for 10 min, and were then centrifuged at 2,000 \(g\) (4°C) for 15 min. A 0.5-ml aliquot was removed from the aqueous phase and quantified by liquid scintillation counting.

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 to a 0.5-ml Fisher microcentrifuge tube containing 1 M benzethonium hydroxide. Liberated \(^{14}\)CO\(_2\) was trapped in the benzethonium hydroxide over 60 min, the microcentrifuge tube containing trapped \(^{14}\)CO\(_2\) was placed in a scintillation vial, and radioactivity was counted.

Calculations and Statistics

The quantity of palmitate (nmol) esterified and oxidized was calculated from the specific activity of the incubation medium [i.e., radiolabeled palmitate (dpm)/total palmitate (nmol)]. Results were analyzed using ANOVA procedures, and a Newman-Keuls 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

Effects of Leptin, IL-6 and TNF-\(\alpha\) on Skeletal Muscle FA Oxidation

Leptin caused a 50% increase in the rate of exogenous FA oxidation \((P < 0.05; \text{Fig. 1A})\). Similarly, IL-6 at a concentration of 5,000 pg/ml stimulated exogenous FA oxidation by 51% \((P < 0.05; \text{Fig. 1A})\). TNF-\(\alpha\) had no effect on exogenous FA oxidation. Endogenous lipid oxidation was increased by leptin (53%, \(P < 0.05\); Fig. 1B) and the highest concentration of IL-6 (54%, \(P < 0.05\); Fig. 1B). TNF-\(\alpha\) did not affect endogenous lipid oxidation.

Combined Effects of Insulin and IL-6, IL-6 and TNF-\(\alpha\), and Leptin and TNF-\(\alpha\) on Skeletal Muscle FA Oxidation

When insulin and IL-6 were both present, IL-6 attenuated insulin’s suppressive effects on exogenous FA oxidation.

Fig. 2. Effect of insulin and insulin + IL-6 on exogenous (A) and endogenous (B) palmitate oxidation, palmitate esterification into diacylglycerol (DAG; C) and triacylglycerol (TAG; D), and total palmitate uptake (E). Soleus muscle strips were incubated in the presence of insulin (10 mU/ml) or insulin + IL-6 (5,000 pg/ml). Dashed line represents control values obtained from the previous experiment. Values are means ± SE. All values are expressed as nmol/g wet wt; \(n = 8–10\) per group. *Significantly different from insulin-treated group, \(P < 0.05\).
(+37%, P < 0.05; Fig. 2A). The combination of IL-6 and TNF-α (Table 1), and leptin and TNF-α (Table 1) had no effect on exogenous FA oxidation. No differences were found in endogenous lipid oxidation with any treatment combination (Fig. 2B and Table 1).

Table 1. Effect of TNF-α on exogenous and endogenous palmitate oxidation in the presence of either IL-6 or leptin

<table>
<thead>
<tr>
<th>Condition</th>
<th>Exogenous Palmitate Oxidation</th>
<th>Endogenous Palmitate Oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>67.8±3.7</td>
<td>12.4±1.0</td>
</tr>
<tr>
<td>IL-6 + TNF-α</td>
<td>61.2±4.1</td>
<td>10.8±2.1</td>
</tr>
<tr>
<td>Leptin</td>
<td>71.9±7.8</td>
<td>12.4±1.2</td>
</tr>
<tr>
<td>Leptin + TNF-α</td>
<td>66.8±6.5</td>
<td>10.0±0.8</td>
</tr>
</tbody>
</table>

All values are means ± SE expressed as nmol/g wet wt; n = 8–10 per group.

Table 2. Effect of IL-6 and TNF-α on total palmitate uptake

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total Palmitate Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>336±25</td>
</tr>
<tr>
<td>Leptin</td>
<td>325±24</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
</tr>
<tr>
<td>50 pg/mL</td>
<td>324±18</td>
</tr>
<tr>
<td>500 pg/mL</td>
<td>333±27</td>
</tr>
<tr>
<td>5,000 pg/mL</td>
<td>346±22</td>
</tr>
<tr>
<td>TNF-α</td>
<td></td>
</tr>
<tr>
<td>50 pg/mL</td>
<td>311±18</td>
</tr>
<tr>
<td>500 pg/mL</td>
<td>298±34</td>
</tr>
<tr>
<td>5,000 pg/mL</td>
<td>315±33</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as nmol/g wet wt; n = 8–10 per group. CON, control.

Effects of Leptin, IL-6 and TNF-α on Palmitate Esterification and Uptake

FA incorporation in DAG was unchanged following treatment with either leptin or IL-6. However, at high concentrations of TNF-α, DAG synthesis was increased by 45% (P < 0.05; Fig. 3A). Leptin reduced FA incorporation into TAG by 19% (P < 0.05; Fig. 3B), whereas neither concentration of IL-6 or TNF-α influenced palmitate esterification to TAG. Total palmitate uptake (esterification + oxidation) was not different between any of the treatments (Table 2).

Combined Effects of Insulin and IL-6, IL-6 and TNF-α, and Leptin and TNF-α on Palmitate Esterification and Uptake

In the presence of IL-6 and insulin, FA incorporation in DAG was not different compared with insulin alone (Fig. 2C). Similarly, when TNF-α was combined with either IL-6 or leptin, no differences were found in DAG synthesis compared with the IL-6 and leptin treatment groups, respectively (Table 3). IL-6 attenuated insulin’s stimulatory effect on esterification of FA to TAG (~22%, P < 0.05; Fig. 2D). There was also a tendency for IL-6 to reduce FA uptake in the presence of insulin (~15%, P = 0.06; Fig. 2E). Addition of TNF-α to either IL-6 (Table 3) or leptin (Table 3) had no effect on TAG esterification or FA uptake compared with IL-6 or leptin treatment alone.

DISCUSSION

In this study, we used the pulse-chase technique in isolated soleus strips to examine the direct effect of IL-6 and TNF-α on skeletal muscle FA metabolism. To our knowledge, this is the first study to provide evidence for the regulation of skeletal
muscle FA metabolism by these cytokines. Several novel observations were made in the current investigation: 1) IL-6 stimulates exogenous and endogenous FA oxidation; 2) TNF-α increases the esterification of FA into the DAG pool; 3) the lipogenic effects of insulin were attenuated by IL-6; and 4) IL-6 and leptin inhibited the TNF-α-induced stimulation of DAG synthesis.

Effect of IL-6 on Skeletal Muscle FA Metabolism

A major finding of the present study was that IL-6 stimulated both exogenous and endogenous FA oxidation. These results are consistent with recent findings reported in humans that infusion of IL-6 increases lipolysis and rates of whole body fat oxidation (36). Taken together, these results suggest that IL-6 is involved in the regulation of skeletal muscle fat metabolism. This is further supported by the observation that, in the presence of insulin, IL-6 attenuated the lipogenic actions of insulin on skeletal muscle FA metabolism. Furthermore, the increase in both exogenous and endogenous FA oxidation in response to IL-6 was of a similar magnitude to that of leptin, a key modulator of skeletal muscle lipid metabolism (22, 34). Because the capacity for FA oxidation is reduced in insulin-resistant states (16) and improvements in insulin sensitivity are associated with enhanced rates of FA oxidation (8), it is possible that IL-6 may have an insulin sensitizing effect on muscle.

The mechanism(s) whereby IL-6 stimulates FA oxidation remains to be elucidated. van Hall et al. (36) suggested that the changes in fat metabolism observed during IL-6 infusion may not be elicited by a direct effect of IL-6, but indirectly via coincidental changes in other hormones such as epinephrine and cortisol. However, the data from the present study strongly point to a direct effect of IL-6 itself on the regulation of skeletal muscle FA metabolism. Furthermore, the presence of mRNA encoding for the IL-6 receptor has been identified in skeletal muscle (40). Recently, it has been suggested that IL-6 may play a role in activating 5'-AMP-activated protein kinase (AMPK) in skeletal muscle (19). AMPK has been proposed to act as a metabolic master switch that stimulates glucose uptake and FA oxidation in skeletal muscle (20). Other adipokynesines that stimulate FA oxidation, such as adiponectin (37) and leptin (21), phosphorylate and activate the α2-catalytic subunit of AMPK (AMPK-α2) in skeletal muscle, suggesting that AMPK is a mediator of adiponectin and leptin's effects on FA metabolism in muscle. However, at the moment there is no direct evidence to suggest that IL-6 activates AMPK. Therefore, future studies are required to elucidate the mechanism(s) by which IL-6 stimulates FA oxidation in muscle.

Effect of TNF-α on Skeletal Muscle FA Metabolism

There is increasing evidence to support a role for TNF-α in modulating lipid metabolism in adipose tissue (26). However, to the best of our knowledge, no study has investigated the effect of TNF-α on FA metabolism in muscle. In this study, we found that TNF-α had no effect on FA oxidation, FA uptake, or esterification of exogenous palmitate into TAG. However, an important finding was that TNF-α caused an increase in FA esterification into the DAG pool. Studies in cell lines have demonstrated that treatment with TNF-α causes a rapid increase in production of DAG (29). The DAG accumulated in response to TNF-α may lead to the activation of acidic sphingomyelinase, which causes the breakdown of sphingomyelin to produce ceramides (17).

FA-derived moieties, such as DAG and ceramide, are thought to play a key role in the pathogenesis of insulin resistance (5). Intramyocellular DAG levels are elevated in a number of models of insulin resistance (14, 27). DAG is a potent activator of some protein kinase C (PKC) isoforms [βII, δ, and θ (14, 27)], which may interfere with insulin signal transduction, particularly via serine phosphorylation of IRS-1 (39). Ceramide content has also been reported to be elevated in muscle from obese insulin-resistant humans (1). Ceramide can inhibit insulin-stimulated glucose transport, glycogen synthesis, and Akt activation (28, 35). In addition there is evidence that increased lipid availability may also cause insulin resistance by activating other serine/threonine kinases, including the inhibitor κB protein kinases in the NF-κB pathway (14) and the c-Jun NH2-terminal kinases [JNK (10)]. Interestingly, TNF-α is a potent physiological inducer of NF-κB and JNK in skeletal muscle cell culture (6). Thus TNF-α-induced insulin resistance may involve impairment of insulin signal transduction via generation of DAG and the subsequent activation of serine/threonine kinases.

Although we observed that TNF-α increased FA incorporation into DAG, we were able to show that this effect was inhibited in the presence of either IL-6 or leptin. Leptin reduces esterification of FA into TAG in both rodent (22) and human skeletal muscle (34). Furthermore, leptin (22) and IL-6 (present study) attenuate insulin’s ability to stimulate TAG esterification. However, IL-6 alone was without effect on esterification of FA into endogenous lipid pools. Thus the mechanism(s) by which IL-6 and leptin oppose the increase in DAG esterification induced by TNF-α is unclear but suggests that leptin and IL-6 may counter the actions of TNF-α.

In conclusion, the results from the present study demonstrate that IL-6 and TNF-α are modulators of skeletal muscle FA metabolism. We provide direct evidence that IL-6 plays a role in regulating skeletal muscle FA metabolism, increasing rates of FA oxidation, and attenuating insulin’s ability to suppress FA oxidation and stimulate lipogenesis. In contrast, TNF-α had no effect on FA oxidation but increased FA incorporation into DAG.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Kerry Mullen.

GRANTS

This study was funded by Natural Sciences and Engineering Research Council of Canada Discovery and Collaborative Health Research grants (D. J. Dyck).

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