IL-6 increases muscle insulin sensitivity only at superphysiological levels

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On the basis of this information, it appears that the acute and short-term effects of transient increases in IL-6 are very different from those associated with long-term elevations of IL-6 caused by conditions that result in chronic inflammation. It has been reported by Kelly et al. (19) that treatment of rat extensor digitorum longus muscle with 120 ng/ml IL-6 results in increased phosphorylation of AMP-activated protein kinase (AMPK). It is well documented that AMPK activation results in an increase in muscle glucose uptake (20, 26, 28), which is followed by an increase in insulin sensitivity (8). Thus it seemed possible that IL-6 might induce an increase in muscle insulin sensitivity by activating AMPK. In this context, it was the purpose of the study to determine the effect of IL-6 on glucose transport and insulin sensitivity in skeletal muscle.

METHODS

Materials. [14C]mannitol was obtained from ICN Radiochemicals (Irvine, CA). 2-Deoxy-[1,2-3H]glucose (2-DG) was purchased from American Radiolabeled Chemicals (St. Louis, MO). The AMPK and anti-phospho-[Thr172]AMPK antibodies were purchased from Cell Signaling (Beverly, MA). Anti-phospho-[Thr308]Akt/protein kinase B (PKB)-α was obtained from Upstate (Lake Placid, NY). Recombinant rat IL-6 was obtained from Pierce (RRIL65). All other chemicals were obtained from Sigma (St. Louis, MO).

Treatment of rats and muscle preparations. Male Wistar rats (Charles River) weighing ~80–120 grams were provided with Purina Rat Chow and water ad libitum. At 5:00 P.M., the evening before an experiment, food was removed. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt) followed by the removal of the soleus and epitrochlearis muscles (13, 45). The epitrochlearis is a small, thin muscle that is well suited for studies of glucose transport; it contains predominantly type IIB (fast-twitch, white) fibers. Soleus muscles consist predominantly of type I (slow-twitch red) fibers. The soleus fibers were split longitudinally into strips before incubation, as described previously (14), to allow adequate diffusion of oxygen and glucose. All protocols were approved by the Animal Studies Committee of Washington University.

Muscle treatments. Following dissection, muscles recovered for 60 min in flasks containing 2 ml of Krebs-Henseleit bicarbonate buffer (KHB) with 8 mM glucose, 32 mM mannitol (recovery medium), and a gas phase of 95% O2–5% CO2 and were maintained at 35°C in a shaking incubator. Following recovery, muscles were incubated in rat serum in the presence or absence of IL-6 at concentrations of 120, 10, or 4 ng/ml for 30 min. The muscles were then rinsed briefly and incubated for 3 h in the recovery medium. Muscles were then transferred to the same medium with or without 60 μU/ml insulin and incubated for 30 min with insulin before glucose transport activity measurement. For measurement of the acute effect of IL-6, muscles were incubated with or without IL-6 for 30 min before measurement of glucose transport.

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Results

Acute effects of IL-6 on glucose transport. As shown in Fig. 1, 30 min of exposure to 120 ng/ml IL-6 resulted in an approximately twofold increase in the rate of 2-DG transport in epitrochlearis muscles. The same treatment with IL-6 had no effect on glucose transport activity in the soleus (Fig. 1).

IL-6 treatment increases insulin sensitivity in epitrochlearis muscles. When epitrochlearis muscles were incubated with 120 ng/ml IL-6 for 30 min and then allowed to recover for 3 h, the acute effect of IL-6 on glucose transport had completely worn off (0.36 ± 0.04 μmol 2-DG·ml⁻¹·20 min⁻¹). Treatment with 60 μU/ml insulin for 30 min following the 3-h recovery resulted in an approximately twofold greater increase in glucose transport activity than occurred in muscles that had not been pretreated with IL-6 (Fig. 2). This concentration of insulin (60 μU/ml) results in ~50% of the maximal effect of insulin on glucose transport in unexercised epitrochlearis muscle preparations. In contrast to the increase in insulin sensitivity in the epitrochlearis muscle, pretreatment with IL-6 did not increase the effect of 60 μU/ml insulin on glucose transport activity in the soleus muscle (Fig. 2).

IL-6 treatment results in AMPK activation. There was no increase in phosphorylation of PKB on Thr308 in response to IL-6 (data not shown), providing evidence that IL-6 does not activate the insulin signaling pathway. Incubation of epitrochlearis muscles with 120 ng/ml IL-6 resulted in a significant increase in AMPK phosphorylation (Fig. 3).

Lack of effect of lower concentrations of IL-6. The decision to use an IL-6 concentration of 120 ng/ml in our initial study design was based on the report by Kelly et al. (19), who used this concentration of IL-6 to stimulate AMPK phosphorylation. However, it appears from a search of the literature that the highest plasma concentration of IL-6 induced by exercise that has been reported is ~120 pg/ml (23), with more usual peak values of ~30 pg/ml (39, 40). As might be expected, IL-6...
attains a higher concentration in muscle interstitial fluid than in plasma. At the same time that plasma IL-6 was 120 pg/ml in the study by Langberg et al. (23), gastrocnemius muscle interstitial IL-6 concentration, measured using microdialysis, was ~500 pg/ml (23). Even if interstitial IL-6 concentration was 10-fold higher in exercised muscles than in plasma, it would be <1% as high as the concentration of IL-6 (120 ng/ml) shown to activate AMPK by Kelly et al. (19) and to increase AMPK phosphorylation, glucose transport, and insulin sensitivity in the present study.

We, therefore, did additional experiments to evaluate the effects of lower IL-6 concentrations. We found that incubation of epitrochlearis muscles with either 4 ng/ml (data not shown) or 10 ng/ml IL-6 did not result in a significant increase in glucose transport activity (Fig. 4A). Using two times the amount of muscle protein and double the antiphospho-AMPK antibody that we normally use, we were able to detect a small increase \( (P < 0.08) \) in AMPK phosphorylation in response to treatment of muscles with 10 ng/ml IL-6. However, the effect of 10 ng/ml IL-6 was small compared with the effect of AICAR, which was used as a positive control (Fig. 4B).

**DISCUSSION**

In light of the extensive evidence that IL-6 is a potent inflammatory cytokine that plays a role in the development of a range of pathologies, it seems surprising that exercise results in increases in plasma IL-6 to levels that are much higher than those seen in individuals with inflammatory diseases (23, 32–34, 40). Exercise has a range of health benefits, and, aside from some skeletal muscle inflammation/damage, strenuous exercise does not appear to have harmful effects in individuals in whom exercise is not contraindicated because of medical problems. There has been much interest in the biological role(s) of the increase in IL-6 during exercise, and data obtained on humans has been interpreted as indicating that, rather than causing insulin resistance, IL-6 enhances insulin-stimulated glucose disposal during exercise (12). On the basis of in vitro studies, this effect has been attributed to activation of AMPK (3, 19). It is well documented that activation of AMPK results in an increase in muscle glucose transport (11, 20, 26, 28) and is followed by an increase in insulin sensitivity (8).

In the first study to show that IL-6 activates AMPK, Kelly et al. (19) used an unphysiologically high concentration of IL-6. Using the same concentration of IL-6 (120 ng/ml), we confirmed their finding of an increase in AMPK phosphorylation. Not surprisingly, in light of the known effects of AMPK activation, this high concentration of IL-6 also resulted in increases in muscle glucose transport and insulin sensitivity in the epitrochlearis muscle. However, a lower concentration of IL-6 (10 ng/ml), which is still many times higher than the concentrations attained during exercise, had no effect on glucose transport. In contrast to our findings, Carey et al. (3) reported recently that 1 ng/ml IL-6 caused a measurable in-
increase in 2-DG uptake and that 10 ng/ml IL-6 caused an approximately twofold increase in 2-DG uptake, whereas 1 and 10 ng/ml IL-6 caused large increases in translocation of GLUT4 to the cell surface in L6 myotubes (3).

We have previously argued that L6 myotubes are not a good model for studying muscle glucose transport because they are only modestly responsive to insulin (16, 42), with increases in glucose transport of 50–100% in response to a maximal insulin stimulus, even in L6 myotubes transfected with mvyc-tagged GLUT4 (25, 29, 38, 41). However, this criticism does not seem valid relative to the L6 myotubes used by Carey et al. (3), which were remarkably responsive to insulin with a fourfold increase in GLUT4 translocation and threefold increase in 2-DG transport in response to insulin, and a fourfold increase in 2-DG transport in response to insulin plus IL-6. We have no explanation for the difference between our findings and those of Carey et al. (3).

Nevertheless, we think it likely that findings obtained on rat skeletal muscle are more relevant to the effects of exercise than those obtained on myotubes and tentatively conclude that, although IL-6 can activate AMPK and muscle glucose transport, as well as increase muscle insulin sensitivity, it does so only at extremely high concentrations that likely have no biological relevance.

In light of the extensive evidence that IL-6 is a potent inflammatory cytokine that appears to play a role in development of a range of chronic pathologies, an obvious question is: Why do the much higher concentrations of IL-6 attained during exercise have no apparent harmful effects? Although our study sheds no light on this question, we offer the speculation that the harmful effects of IL-6 require continuous exposure of tissues to elevated levels of IL-6 for prolonged periods, whereas the elevations of IL-6 induced by exercise are brief.

In conclusion, we interpret the results of this study as evidence that IL-6, at the concentrations attained during exercise, does not acutely increase muscle glucose transport or insulin sensitivity.

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