Exercise-mediated IL-6 signaling occurs independent of inflammation and is amplified by training in mouse adipose tissue

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Exercise-mediated IL-6 signaling occurs independent of inflammation and is amplified by training in mouse adipose tissue. J Appl Physiol 119: 1347–1354, 2015. First published October 1, 2015; doi:10.1152/japplphysiol.00551.2015.—The purpose of this investigation was to determine whether exercise-induced increases in adipose tissue interleukin 6 (IL-6) signaling occurred as part of a larger proinflammatory response to exercise and whether the induction of IL-6 signaling with acute exercise was altered in trained mice in parallel with changes in the IL-6 receptor complex. Sedentary and trained C57BL/6J mice were challenged with an acute bout of exercise. Adipose tissue and plasma were collected immediately and 4 h afterward and analyzed for changes in indices of IL-6 signaling, circulating IL-6, markers of adipose tissue inflammation, and expression/content of IL-6 receptor and glycoprotein 130 (gp130) in untrained mice. IL-6 mRNA increased immediately after exercise, and increases in indices of IL-6 signaling were increased 4 h after exercise in epididymal, but not inguinal adipose tissue. This occurred independent of increases in plasma IL-6 and alterations in markers of inflammation. When compared with untrained mice, in trained mice, acute exercise induced the expression of gp130 and IL-6 receptor alpha (IL-6Rα), and training increased the protein content of these. Acute exercise induced the expression, and training increased the protein content of glycoprotein 130 and IL-6Rα and was associated with a more rapid increase in markers of IL-6 signaling in epididymal adipose tissue from trained compared with untrained mice. The ability of exogenous IL-6 to increase phosphorylation of STAT3 was similar between groups. Our findings demonstrate that acute exercise increases IL-6 signaling in a depot-dependent manner, likely through an autocrine/paracrine mechanism. This response is initiated more rapidly after exercise in trained mice, potentially as a result of increases in IL-6Rα and gp130.

Exercise; adipose tissue; IL-6; mice; inflammation

INTERLEUKIN 6 (IL-6) IS A pleiotropic cytokine with reputed metabolic and immuno-modulating properties (29). IL-6 classically signals through binding to the IL-6 receptor alpha subunit (IL-6Rα). This results in the recruitment and homodimerization of glycoprotein 130 (gp130) and the subsequent activation of downstream effectors such as STAT3. In the context of exercise, IL-6 has traditionally been viewed as an endocrine factor secreted from skeletal muscle (30). However, an underappreciated aspect of IL-6 biology is that it is also rapidly induced (16) and secreted from adipose tissue (22) with exercise. The specific role of IL-6 in regulating adipose tissue immuno-metabolism is a hotly debated topic with both pro- and anti-inflammatory properties being ascribed to this cytokine [as reviewed in (19)]. In this context it is unclear whether exercise-induced increases in adipose tissue IL-6 occur as part of a larger proinflammatory response to acute exercise, as has previously been reported by some (32) but not all (6), or whether it occurs independent of changes in inflammation. Exercise training confers a protective effect in adipose tissue against an acute inflammatory stress induced pharmacologically via the beta 3 adrenergic receptor agonist CL 316,243 (7) or via an endotoxic challenge such as lipopolysaccharide (28). Interestingly, Keller et al. (17) reported increases in IL-6 receptor expression in skeletal muscle with exercise training. In contrast to the protective effects against inflammatory challenges, elevated expression of the IL-6 receptor may suggest an augmented capacity for IL-6 signaling with exercise in the trained state. To the best of our knowledge the effects of exercise training on components of the IL-6 receptor complex (i.e., IL-6Rα, gp130) and how this could influence the acute effects of exercise on IL-6 signaling in adipose tissue has not been studied.

The purpose of the present investigation was to examine the effects of exercise on indices of adipose tissue IL-6 signaling in sedentary and trained conditions. Specifically, we wanted to determine whether exercise-induced increases in adipose tissue IL-6 occurred in parallel with induction of markers of inflammation. The second aim of this paper was to examine the effects of exercise on indices of IL-6 signaling in adipose tissue with exercise training. We hypothesized that a single, nonexhaustive bout of treadmill running would increase markers of IL-6 signaling independent of inflammation. We further postulated that exercise training would increase the content of IL-6Rα and gp130, and that this would be associated with an enhanced response of IL-6 signaling to acute exercise in adipose tissue from trained mice.

MATERIALS AND METHODS

Materials. Nonesterified free fatty acid (NEFA) kits (NEFA-HR kits) were from Wako Chemicals (Richmond, VA). Molecular weight marker, reagents, and nitrocellulose membranes for SDS-PAGE were purchased from Bio-Rad (Mississauga, ON, Canada). ECL Plus was a product of Amersham Pharmacia Biotech (Arlington Heights, IL). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit and goat anti-mouse IgG secondary antibodies were from Jackson ImmunoResearch Laboratories (West Grove, PA). Antibodies against total Janus Kinase (JNK) (Cat. No. 9252), phospho JNK Thr183/Tyr185 (Cat. No. 4671), total p38 MAPK (Cat. No. 9211), phospho p38 MAPK Thr180/Tyr182 (Cat. No. 9212), total STAT3 (Cat. No. 9139), phospho Tyr705 STAT3 (Cat. No. 9131), and gp130 (Cat. No. 2213)
were purchased from Cell Signaling (Danvers, MA). An antibody against IL-6Rα (Cat. No. 1211) was purchased from Sigma-Aldrich (St. Louis, MO).

Animals. Male C57BL/6J mice (~10 wk old) were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed one per cage. Animals had free access to water and standard rodent chow (7004-Teklad S-2335 Mouse Breeder Sterilizable Diet; Teklad Diets Harlan Laboratories, Madison WI) and were maintained on a 12-h:12-h light:dark cycle. All protocols were approved by the University of Guelph Animal Care Committee and met the guidelines of the Canadian Council on Animal Care (8). The number of mice per experiment is listed in the figure legends.

Acute exercise in untrained mice. Mice were acclimated to motor-ized treadmill running for 10 min at 15 m/min at a 5% incline on 2 successive days. Approximately 48 h after the last acclimation bout, mice ran for 2 h at 15 m/min at a 5% incline. Food was removed from all mice 1 h prior to the start of exercise. Immediately (0 h) or 4 h after exercise mice were anesthetized with a weight-adjusted bolus injection of sodium pentobarbital (5 mg/100 g body wt), then epididymal and inguinal adipose tissue dissected and immediately frozen in liquid nitrogen and stored at −80°C. Intrathoracic blood was then collected, centrifuged for 10 min (5,000 g, 4°C), and plasma was collected and stored at −80°C. Mice in the 4-h postexercise groups were given free access to water but not food. The order in which mice were euthanized was rotated to minimize differences in circadian variation between the four groups.

Acute exercise in trained mice. As we have described previously (7), mice were trained by treadmill running 1 h/day, 5 days/wk for 4 wk. This training protocol leads to expected increases in markers of skeletal muscle mitochondrial content and reductions in adipose tissue mass (7). In an effort to match the relative exercise intensity for the acute exercise challenge in the trained mice to what we used in our first set of experiments in the untrained mice, we used running speed as a surrogate measure of exercise intensity (11). Maximal running speed was determined in a running test adapted from that described by Tuti et al. (36). Mice began running at a speed of 10 m/min at a 5% incline, and the speed was increased every 3 min by 3 m/min until mice could no longer maintain the pace for 3 min. The maximum running speed was ~30 and 38 m/min for sedentary and trained mice, respectively. This test occurred during the last week of training. Forty-eight hours after the last bout of training mice were exercised for 2 h at 19 m/min on a 5% incline. This running speed was chosen to approximate the same relative intensity of exercise as in the untrained mice (i.e., ~50% of maximal speed). Immediately and 4 h after the acute bout of exercise, trained mice were anesthetized with sodium pentobarbital and tissue was collected as described above.

Intraperitoneal IL-6 injections in sedentary and trained mice. Approximately 48 h after the last bout of exercise, sedentary and exercise trained mice were injected with a weight-adjusted dose of recombinant murine IL-6 (3 ng/g body wt ip) or an equivalent volume of sterile saline. This dose of IL-6 has been shown to lead to increases in circulating IL-6 similar to those observed during exercise (5). Adipose tissue was harvested 15 min after injection as described above.

Plasma NEFA and IL-6. Plasma NEFA and IL-6 were concentra-tions were analyzed using commercially available kits as we have described in detail previously (23).

Western blotting. Samples were homogenized (FastPrep-24; MP Biomedicals, Santa Ana, CA) in three volumes of cell lysis buffer supplemented with phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Sigma-Aldrich). Homogenized samples were centrifuged at 4°C (10 min at 5,000 g), the infranatant was collected, and protein content was determined using a bicinchinonic acid assay (35). Equal concentrations of protein were separated on 10% SDS-PAGE gels and then transferred onto nitrocellullose membranes using a wet transfer technique at 200 mA/transfer unit. Membranes were blocked in Tris-buffered saline/0.1% Tween (TBST) with 5% nonfat dry milk for 1 h then incubated in TBST/5% BSA supplemented with the appropriate primary antibody (1:1,000 dilution) at 4°C with gentle agitation overnight. Following incubation in primary antibody, membranes were rinsed with TBST and incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. Secondary antibodies were diluted in TBST with 1% nonfat dry milk. Signals were detected using enhanced chemiluminescence and were subsequently quantified by densitometry using a FluorChem HD imaging system (Alpha Innotech, Santa Clara, CA). The phospho signal was expressed relative to total, or in the case of IL-6Rα and gp130, ponceau.

RT-PCR. Changes in mRNA expression were determined using RT-quantitative PCR as described in detail previously by our laboratory (38, 39). Briefly, RNA was isolated from adipose tissue using an RNeasy kit according to the manufacturer’s instructions. Complementary DNA was synthesized from total RNA (1 ug) using SuperScript II Reverse Transcriptase, 2-deoxynucleotide 5'-triphosphate, and random primers. RT-PCR was completed using a 7500 Fast Real-Time PCR system (Applied Biosystems). The ΔΔCT method (21) was used to calculate fold differences in gene expression with GAPDH or Polyr2 used as a housekeeping gene.

Statistical analysis. Comparisons between two groups were made using unpaired, two-tailed t-tests. Differences in gene expression over time were determined using a one-way ANOVA, whereas signaling and NEFA levels were compared using a two-way ANOVA. When indicated, ANOVA was followed by least significant differences post hoc analysis. In some cases data were logarithmically transformed to ensure equal variance among groups. Data are expressed as means ± SE and significance was set at P < 0.05.

RESULTS

An acute bout of exercise increases markers of IL-6 signal-ing, but not inflammation in mouse adipose tissue. To initially characterize the effects of exercise on markers of inflammation we measured the mRNA expression of IL-6, SOCS3, IL-1β, and TNFα in epididymal and inguinal adipose tissue. Adipose tissue fatty acid release has been suggested to play a role in the induction of adipose tissue inflammation (26), and thus we chose to examine alterations in markers of inflammation in two fat depots that display differences in lipolytic responsiveness (epididymal > inguinal) in vivo (31). As shown in Figure 1A, an acute bout of exercise increased IL-6 mRNA expression by approximately threefold immediately after exercise, and it remained elevated 4 h following exercise in epididymal adipose tissue. SOCS3, a transcriptional target of IL-6 signaling (20, 37), was significantly increased 4 h after exercise, whereas expression of IL-1β and TNFα were not increased with exercise.

IL-6 signals through the JAK/STAT signaling pathway and we have recently shown that IL-6 directly increases STAT3 phosphorylation in cultured mouse adipose tissue (38). To determine a potential functional correlate to the induction of IL-6 in adipose tissue following exercise, we measured STAT3 phosphorylation 4 h after exercise. This time point coincided with the increase in SOCS3 expression, a canonical IL-6 target
gene (20, 37). Four hours following exercise the phosphorylation of STAT3 was increased by approximately 2.5-fold in epididymal adipose tissue (Fig. 1A, right). In contrast to STAT3, but consistent with our gene expression data, an acute bout of exercise did not lead to increases in the phosphorylation of p38 or JNK, kinases that are typically activated in proinflammatory conditions (2, 12, 34).

To ascertain whether depot-specific differences existed in regards to the induction of gene expression and proinflammatory kinase activation with exercise, we assessed changes in markers of inflammation and IL-6 signaling in inguinal subcutaneous adipose tissue. As shown in Figure 1B, we found no changes in expression of IL-6, SOCS3, TNFα, or IL-1β either immediately or 4 h following exercise. Similarly, we did not detect any changes in the phosphorylation of STAT3, p38, or JNK (Fig. 1B, right).

Others have reported an effect of food restriction on inflammatory markers (3). Although we removed food from all mice at the same time and rotated the time of euthanasia for the sedentary animals to minimize circadian variability between groups, we wanted to ensure that subtle differences in the length of fasting was not playing a role in the observed increases in IL-6 and SOCS3. To accomplish this, mice were fasted for 8 h, and epididymal adipose tissue was harvested.
The mRNA expression of IL-6 (fed 1.15 ± 0.23, fasted 0.48 ± 0.12, n = 6/group) and SOCS3 (fed 1.08 ± 0.21, fasted 0.74 ± 0.09, n = 6/group) were not increased in epididymal adipose tissue from fasted mice.

Exercise increases the mRNA expression and protein content of IL-6Rα and gp130. A single bout of exercise has previously been shown to upregulate the expression of the IL-6Rα in skeletal muscle (17). To the best of our knowledge the effects of exercise on components of the IL-6 receptor complex in adipose tissue remain unknown. Given this we examined the effects of an acute bout of exercise on the expression of IL-6Rα and gp130 in epididymal adipose tissue. As shown in Figure 2A, IL-6Rα and gp130 mRNA expression were increased by approximately threefold and 1.5-fold, respectively, 4 h after a single bout of exercise. In light of these increases in receptor expression, we next considered the effect of repeated bouts of exercise on the protein content of these receptors. Four weeks of exercise training led to increases in both IL-6Rα and gp130 protein content in epididymal adipose tissue (Fig. 2B).

**Acute exercise causes a more rapid increase in markers of IL-6 signaling in adipose tissue from trained compared with sedentary mice.** Given the increase in the content of gp130 and IL-6Rα in adipose tissue with training, we next sought to determine whether this was associated with alterations in the response of IL-6 signaling to an acute bout of exercise. In an effort to match relative exercise intensities between sedentary and trained animals, mice ran at ~50% of their maximum running speed, which equated to 15 m/min and 19 m/min in the sedentary and trained mice, respectively. As shown in Figure 3A, induction of IL-6 in epididymal adipose tissue from trained and sedentary mice was similar both immediately and 4 h following an acute bout of exercise. Conversely, SOCS3 mRNA expression was increased to a greater extent immediately after exercise in epididymal adipose tissue from trained compared with sedentary mice (Fig. 3B). In keeping with these findings, STAT3 phosphorylation was increased immediately after exercise in epididymal adipose tissue from trained mice, but it did not immediately increase in sedentary mice (Fig. 3C). By 4 h postexercise STAT3 phosphorylation had returned to pre-exercise levels in adipose tissue from trained mice (data not shown).

**Markers of lipolysis are similar in response to an acute bout of exercise in sedentary and exercise-trained mice.** Lipolysis has been previously shown to play a role in induction of IL-6 signaling in adipose tissue (26). Consequently, we wanted to determine whether the more rapid increase in indices of IL-6 signaling could be related to alterations in markers of lipolysis. As shown in Figure 4, hormone-sensitive lipase (HSL) phosphorylation in epididymal adipose tissue (Fig. 4A) and plasma NEFAs (Fig. 4B) were not different between groups.

**Circulating levels of IL-6 are not different between sedentary and exercise trained mice.** Exercise increases circulating IL-6 (9), and therefore it was of interest to determine whether exercise-induced increases in IL-6 were different between sedentary and trained mice. In untrained mice an acute bout of exercise led to a >60 pg/ml increase in plasma IL-6, although this failed to reach significance due to the high variability among animals. In trained mice, an acute bout of exercise did not increase circulating IL-6 levels (Table 1). Four hours after exercise, plasma IL-6 levels were similar to sedentary levels in both untrained and trained mice (data not shown).

**Effects of exercise training on exogenous IL-6 signaling.** Because training-induced increases in IL-6Rα and gp130 were associated with a more rapid exercise-induced increase in adipose tissue IL-6 signaling, we wanted to determine whether a similar relationship existed when mice were presented with an exogenous IL-6 challenge. Interestingly, phosphorylation of STAT3 was activated to a similar extent in epididymal adipose tissue from both sedentary and trained mice 15 min after an injection of recombinant murine IL-6 (Fig. 5).

**DISCUSSION**

The effect of exercise training on modulation of adipose tissue inflammation has traditionally been investigated in models of obesity and insulin resistance. In these studies, markers of inflammation typically track with changes in adipose tissue mass (4, 13–15). In the current investigation, we characterized the induction of IL-6 expression and signaling in concert with changes in markers of inflammation in response to an acute bout of exercise.
bout of exercise. We have shown sequential increases in the expression of IL-6 and SOCS3 mRNA, and these findings are consistent with previous work demonstrating IL-6-mediated increases in SOCS3 in cultured adipocytes/adipose tissue (20, 37). These results, together with other results showing increases in STAT3 phosphorylation, would suggest an induction of IL-6 signaling in epididymal adipose tissue following exercise. Interestingly, the increases in IL-6 signaling occurred more rapidly in adipose tissue from trained mice compared with untrained mice in parallel with increases in gp130 and IL-6Rα protein content.

It is our current hypothesis that an increase in adipose tissue IL-6 signaling is likely occurring via increases in adipose tissue-derived IL-6, not circulating IL-6. Notably, the increases in indices of IL-6 signaling (i.e., SOCS3 mRNA, STAT3 phosphorylation) following acute exercise in both untrained (4 h after exercise) and trained (immediately after exercise) mice occurred at a time point when circulating IL-6 levels were not appreciably elevated. Given previous reports that measures of IL-6 concentration surrounding human subcutaneous adipose tissue were found to be orders of magnitude higher than exercise-induced circulating IL-6 levels (27), it is tempting to speculate that exercise-mediated increases in circulating IL-6 would be of little consequence to IL-6 signaling in adipose tissue. However, in the current study, we found that an injection of IL-6 led to a robust increase in STAT3 phosphorylation in epididymal adipose tissue, a finding that is consistent with a recent report showing increases in the phosphorylation of STAT3 in inguinal adipose tissue following IL-6 injections (18). These findings provide evidence that increases in circulating IL-6 are in fact sufficient to induce IL-6 signaling in adipose tissue and perhaps point toward key species-specific differences in IL-6 biology. Although we cannot discount the possibility that transient increases in circulating IL-6 that were missed in the current study precede, and perhaps drive increases in adipose tissue IL-6 signaling, or that an IL-6-independent mechanism is activating canonical IL-6 pathways, our data are temporally consistent with the notion that exercise-induced increases in IL-6 signaling in adipose tissue is potentially mediated through a paracrine/autocrine mechanism.

In the current study we show clear depot-specific effects of exercise. These results are in line with previous work from our laboratory in which induction of IL-6 and SOCS3 by CL 316,243 was approximately 10-fold less in inguinal compared with epididymal mouse adipose tissue (7). The attenuated response of inguinal adipose tissue could be related to differences in lipolysis (31) because the attenuation of fatty acid release by the HSL inhibitor BAY 59–9435, blunts the proinflammatory effects of CL 316,243 in mouse adipose tissue in vivo (26). In contrast to the present study, which used lean mice, we recently reported that an acute bout of exercise robustly induces markers of IL-6 signaling in inguinal adipose tissue from obese, insulin-resistant mice fed a high-fat diet (24). Although speculative, these differences could be due to alterations in adipose tissue lipid handling and/or increases in macrophage infiltration/polarization that are present with diet-induced obesity.

Having assessed the effects of acute exercise in untrained mice we next wanted to determine whether exercise training would 1) alter the content of IL-6Rα and gp130, and 2) whether this would augment the effects of exercise on adipose tissue IL-6 signaling. We demonstrated that an acute bout of exercise induced mRNA expression, whereas exercise training increased the protein content of IL-6Rα and gp130. Interestingly, although exercise increased the mRNA expression of IL-6 to a similar extent in epididymal adipose tissue from untrained and trained mice, both expression of SOCS3 mRNA and phosphorylation of STAT3 were elevated immediately after exercise in trained mice, whereas in untrained mice they were not. Although changes in the rate of lipolysis can influence the expression of IL-6 (26), neither HSL phosphorylation nor levels of circulating free fatty acids were different between sedentary and trained mice immediately after exercise. Although these end points are admittedly indirect indices of lipolysis, they suggest that rapid induction of IL-6 signaling with training is likely not secondary to changes in fatty acid release.

Although these findings suggest an enhancement of IL-6 signaling with training in response to an acute bout of exercise, phosphorylation of STAT3 was increased to a similar extent in trained and untrained mice injected with IL-6. These discrepancies could be due to differences in how rapidly IL-6 increases (either systemically or locally), or the different hormonal milieus (catecholamines, etc.) present with exercise.

Fig. 3. Exercise training leads to a more rapid induction of exercise-induced IL-6 signaling in epididymal adipose tissue. Mice were treadmill-trained for 1 h/day, 5 days/wk for 4 wk. Forty-eight hours after the last bout of training, untrained and trained mice ran (15 m/min untrained, 19 m/min trained, 5% incline) on a treadmill for 2 h and epididymal adipose tissue was harvested either immediately (0 h) or 4 h after exercise to determine IL-6 mRNA (A), SOCS3 mRNA (B), or STAT3 (C) phosphorylation. In (A) and (B), data are presented as fold increases compared with sedentary mice in the same group. Data are presented as means ± SE for 6–8 mice/group. *P < 0.05 compared with untrained in (B) and no exercise in (C). C, top right: representative Western blots.
Table 1. Plasma IL-6 levels in sedentary and previously trained mice following an acute bout of exercise

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sedentary, No Previous Exercise</th>
<th>Sedentary, Plus Previous Exercise</th>
<th>Trained, No Previous Exercise</th>
<th>Trained, Plus Previous Exercise</th>
</tr>
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<tbody>
<tr>
<td>Plasma IL-6, pg/ml</td>
<td>2.9 ± 0.7</td>
<td>71.2 ± 41.2</td>
<td>2.4 ± 0.3</td>
<td>3.2 ± 1.8</td>
</tr>
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Data are means ± SE for 6–7 mice/group. IL-6, interleukin-6.

We have previously shown that exercise training attenuates the induction of IL-6 signaling by the lipolytic agent CL 316,243 (7). Given that CL 316,243 exclusively targets beta-3 adrenergic receptors (10), it is not surprising that the effects of this compound do not mirror those of an acute exercise challenge. In particular, the transient surge in circulating catecholamines with exercise stimulates both the lipolytic beta (1–3) and antilipolytic alpha-adrenergic receptors [as reviewed in (27)], thus tailoring the release of lipids to match the demands of the environment. The relatively unchecked stimulation of lipolysis by CL 316,243 through the specific activation of beta-3 adrenergic receptors therefore represents a unique and nonphysiological metabolic challenge. This is reflected in the ability of CL 316,243 to induce the expression of proinflammatory genes (33), which is not observed following acute exercise.

The role of IL-6 signaling in adipose tissue remains controversial. Although traditionally considered a proinflammatory cytokine, there is mounting evidence to suggest that this cytokine is involved in the anti-inflammatory response [as reviewed in (19)]. For example, the macrophage-specific deletion of IL-6Rα leads to a worsening of high-fat diet-induced insulin resistance and a more proinflammatory phenotype in mouse adipose tissue (25). These findings suggest that IL-6 is perhaps induced as a compensatory response to inflammatory challenges as a means to dampen or resolve inflammation. In the current study, induction of IL-6 appears not to be occurring in response to an inflammatory stress because traditional markers of inflammation (i.e., JNK and p38 phosphorylation, TNFα, and IL-1β mRNA) were not increased. These findings pose the question as to the physiological role of increases in IL-6 signaling postexercise. Although this is merely speculative, perhaps the activation of IL-6 signaling in adipose tissue serves to promote or maintain the release of fatty acids. From a teleological perspective, an enhancement of IL-6 signaling postexercise could be beneficial in providing fatty acids to liver compared with injections. It should be noted that we had previously shown that STAT3 phosphorylation was reduced in epididymal adipose tissue immediately after exercise (38). This discrepancy could be due to differences in the duration of exercise (90 vs. 120 min) or perhaps the method that was used to kill the mice [cervical dislocation in (38) compared with exsanguination following tissue removal]. Regardless of the specific reason, both studies clearly show that IL-6 signaling is not activated immediately following exercise in adipose tissue from untrained mice.

![Image](image-url)

**Fig. 4.** Indices of lipolysis are similar following an acute bout of exercise in sedentary and trained mice. Mice ran on a motorized treadmill (15 m/min untrained, 19 m/min trained, 5% incline) for 2 h and epididymal adipose tissue and plasma were harvested immediately afterward to determine hormone-sensitive lipase (HSL) phosphorylation (A) and plasma nonesterified free fatty acid (NEFA) (B). Data are presented as means ± SE for 6–8 samples/group. A, top right: representative Western blots. *P < 0.05 compared with nonexercised mice in the same group.

![Image](image-url)

**Fig. 5.** STAT3 phosphorylation is increased to a similar extent in epididymal adipose tissue immediately after exercise (38) and plasma were harvested immediately afterward. Data are presented as means ± SE for 6–7 mice/group. IL-6, interleukin-6.
and skeletal muscle to be oxidized as a fuel source while sparing glucose for glycogen synthesis. Of note, liver glycogen levels begin to replete much more rapidly following exercise in trained compared with untrained rats (1), a finding that mirrors the more rapid induction of IL-6 signaling in trained mice in the current study. Although we have not been able to demonstrate a direct effect of IL-6 on adipose tissue lipolysis ex vivo (38), it remains a possibility that IL-6 could be working synergistically with other neuroendocrine factors to facilitate this process. However, if this were the case, then one would expect to observe differences in HSL phosphorylation in conditions of elevated IL-6 signaling (i.e., trained compared with untrained immediately after exercise), which we did not observe in the current study. If IL-6 does in fact modulate adipose tissue lipolysis, then perhaps it is doing so through a mechanism independent of HSL, or there is a lag between the activation of IL-6 signaling and the potentiation of lipolysis. Clearly, this is an area that requires further investigation.

In summary, we have provided novel data demonstrating depot-specific, exercise-induced increases in adipose tissue IL-6 signaling independent of inflammation. We have further shown that activation of IL-6 signaling occurs more rapidly in adipose tissue from trained mice when exercised at the same relative running speed as untrained animals, and is associated with increases in the protein content of IL-6Rα and gp130. We speculate that increases in adipose tissue IL-6 signaling following exercise could play a role in the provision of lipids to muscle and liver.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


