Endurance training partially reverses dietary-induced leptin resistance in rodent skeletal muscle

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Steinberg, Gregory R., Angela C. Smith, Sam Wormald, Patrick Malenfant, Cheryl Collier, and David J. Dyck. Endurance training partially reverses dietary-induced leptin resistance in rodent skeletal muscle. Am J Physiol Endocrinol Metab 286: E57–E63, 2004; 10.1152/ajpendo.00302.2003.—Leptin acutely stimulates skeletal muscle fatty acid (FA) metabolism in lean rodents and humans. This stimulatory effect is eliminated following the feeding of high-fat diets in rodents as well as in obese humans. The mechanism(s) responsible for the development of skeletal muscle leptin resistance is unknown; however, a role for increased suppressor of cytokine signaling-3 (SOCS3) inhibition of the leptin receptor has been demonstrated in other rodent tissues. Furthermore, whether exercise intervention is an effective strategy to prevent or attenuate the development of skeletal muscle leptin resistance has not been investigated. Toward this end, 48 Sprague-Dawley rats (175–190 g; ~2–3 mo of age) were fed control or high-fat (60% kcal) diets for 4 wk and either remained sedentary or were treadmill trained. In control diet-fed animals that remained sedentary (CS) or were endurance trained (CT), leptin stimulated FA oxidation (CS +32 ± 15%, CT +30 ± 17%; P < 0.05), suppressed triacylglycerol (TAG) esterification (CS −17 ± 7%, CT −24 ± 8%; P < 0.05), and reduced the esterification-to-oxidation ratio (CS −19 ± 13%, CT −29 ± 10%; P < 0.001) in soleus muscle. High-fat feeding induced leptin resistance in the soleus of sedentary rats (FS), whereas endurance exercise training (FT) restored the ability of leptin to suppress TAG esterification (~19 ± 9%, P = 0.038). Training did not completely restore the ability of leptin to stimulate FA oxidation. High-fat diets stimulated SOCS3 mRNA expression irrespective of training status (FS +451 ± 120%, P = 0.024; FT +381 ± 141%, P = 0.023). Thus the development of skeletal muscle leptin resistance appears to involve an increase in SOCS3 mRNA expression. Endurance training was generally effective in preventing the development of leptin resistance, although this did not appear to require a decrease in SOCS3 expression. Future studies should examine changes in the actual protein content of SOCS3 in muscle and establish whether aerobic exercise is also effective in treating leptin resistance in humans.

suppressor of cytokine signaling-3; tracer; triacylglycerol; glycerogen; treadmill running

LEPTIN ACUTELY INCREASES FATTY ACID (FA) OXIDATION WHILE REDUCING TRIACYLGLYCEROL (TAG) ESTERIFICATION IN BOTH RODENT (36, 37, 44) AND HUMAN SKELETAL MUSCLE (46). THIS EFFECT IS MEDITATED, AT LEAST IN PART, BY THE PHOSPHORYLATION AND SUBSEQUENT ACTIVATION OF THE AMP-ACTIVATED PROTEIN KINASE (AMPK) (34). AMPK REGULATES FA OXIDATION AND ESTERIFICATION BY PHOSPHORYLATING AND DEACTIVATING ACETYL-COO CARBOXYLASE (34) AND GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE (GPAT) (35), RESPECTIVELY. CHRONICALLY, LEPTIN ALSO DECREASES THE ABUNDANCE OF THE FA TRANSPORTERS FATTY ACID TRANSLOCASE (FAT/CD36) AND PLASMA MEMBRANE FATTY ACID-BINDING PROTEIN (FABPPm) IN THE SARCOLEMMA AND CONSEQUENTLY THE TRANSPORT OF FA INTO THE CELL (45).

Skeletal muscle from obese humans exhibits an accumulation of lipid, including intramuscular triacylglycerol (TAG) (32, 43) and long-chain fatty acyl-CoA (21). This is likely due to a combination of several mechanisms, including increased expression of FA transporters and transport rate (Bonen A, Steinberg GR, Dyck DJ, and Heigenhauser GJ, unpublished observations), increased esterification (46), and an unaltered or reduced rate of FA oxidation (i.e., no compensatory increase) (22, 26, 46). Despite the high concentration of circulating leptin that characterizes human obesity (31), there is clearly a dysregulation of FA metabolism in skeletal muscle that is strongly associated with the development of insulin resistance in this tissue (33). Therefore, it is possible that, during the development of human obesity, skeletal muscle becomes resistant to leptin, contributing to the accumulation of intramuscular lipids [TAG, diacylglycerol (DAG), long-chain fatty acyl-CoA]. We (44, 46) have recently provided evidence for this in high-fat-fed rats as well as in moderately obese humans. Leptin resistance in tissues such as the liver and hypothalamus has also been noted following high-fat feeding in rodents (17, 27, 28, 50). Furthermore, published clinical studies in humans have had poor success in enhancing weight loss subsequent to leptin administration, with very high dosages being required to elicit an effect (19). This also strongly supports the hypothesis that overweight humans become leptin resistant. Thus it is of paramount importance to further examine the metabolic basis of the development of leptin resistance as well as to determine whether particular lifestyle strategies, such as aerobic exercise, play a role in modulating leptin sensitivity.

The mechanism(s) that leads to leptin resistance in skeletal muscle is unknown. Several studies have demonstrated that high-fat diets do not alter leptin receptor protein expression (7, 13), suggesting that the cause of resistance lies downstream. Several postreceptor inhibitors of leptin signaling have been identified, including SH2-containing phosphatase; protein inhibitor of activated STAT; and, most notably, a member of the family of suppressors of cytokine signaling, SOCS3. The role of SOCS3 in leptin signaling was first identified in the hypothalamic nuclei of leptin-resistant mice (6), where it potently
inhibits leptin signaling by suppressing STAT3 activation (2, 5, 14, 15). Ad libitum feeding of high-fat diets to rats concurrently induces obesity and increases SOCS3 mRNA and protein expression in white adipose tissue, and its overexpression in pancreatic islets prevents the lipopenic effects of leptin (49). The message (mRNA) for SOCS3 has previously been detected in muscle (29, 51); however, the effect of high-fat diets on SOCS3 mRNA expression in skeletal muscle has not been investigated.

Despite the obvious physiological importance of leptin resistance, the ability to prevent or reverse its development has been relatively unexamined. Dietary modification, such as the ingestion of ω-3 fatty acids, as well as endurance training, is known to improve skeletal muscle insulin sensitivity. However, the role of endurance training as a modulator of leptin’s actions has been completely unexamined. Several studies have demonstrated that skeletal muscle insulin resistance induced by high-fat feeding, or as observed in human obesity, can be at least partially reversed with endurance training independently of changes in body mass (23, 25). Furthermore, endurance-trained humans possess a greater capacity to oxidize FA, despite lower concentrations of circulating leptin (20, 41), indirectly suggesting that endurance training might improve leptin sensitivity.

Therefore, in this study we tested the hypotheses that endurance training would prevent/attenuate the development of leptin resistance induced by a high-fat diet and that SOCS3 mRNA expression would be related to the development of skeletal muscle leptin resistance in rats. To test this hypothesis, we incubated isolated soleus strips in the presence or absence of leptin and monitored FA metabolism with labeled palmitate. In addition, end point determinations of SOCS3 mRNA were made, as well as of blood leptin, insulin, glucose, and FA as general indicators of systemic changes in response to the diet and training protocols. Finally, we also determined the effect of diet and training on muscle glycogen and TAG stores, as well as glycerol phosphate acyltransferase activity in muscle, which is an important determinant of muscle TAG storage.

METHODS

Animals. Upon arrival, female Sprague-Dawley rats of normal estrous cycle status, weighing 175–190 g (Charles River Laboratories, St. Constant, QC, Canada) were assigned to individual cages in a controlled environment with a reverse 12:12-h light-dark cycle. Animals were fed Purina rat chow ad libitum for a 7-day acclimation period. Animals were then assigned to one of four experimental conditions: control diet sedentary (CS), high-fat diet sedentary (FS), control diet trained (CT) or high-fat diet trained (FT). For the majority of the metabolic parameters to be measured, there was an anticipated variance of ~30–35%, requiring a sample size of 12 to detect differences of ~20% or greater between group means. Thus 12 animals were used in each condition. The composition and preparation of the control and high-fat diets have been previously described. Briefly, the control diet was 12% kcal from fat (derived from safflower oil), 16% kcal from protein and 72% kcal from carbohydrate, whereas the high-fat diet was a safflower oil-based diet consisting of 60% kcal from fat; 16% kcal from protein, and 24% kcal from carbohydrate. To discount the possibility that alterations in leptin sensitivity were due to dietary or training-induced changes in body mass, high-fat-fed and endurance-trained rats were pair-fed with their respective controls, which were fed ad libitum. After the 4-wk diet/training protocol, all rats were fasted overnight before any experimental procedures. Because all rats were overnight fasted, it is assumed that any noted metabolic differences between groups were due to diet and/or training. Food intake was similar in all groups, and there were no significant differences in body mass among the groups by the end of the study (Table 1).

Training. Rats were trained with a protocol that we have previously demonstrated to induce significant improvements in markers of aerobic capacity in skeletal muscle (11). Briefly, animals were run on a treadmill at 21 m/min on a 15% incline 5 days/wk, beginning at 20 min/day, with the duration gradually increasing to 2 h/day by the end of the 2nd wk. Trained rats continued to run at this pace and duration for the following 2 wk. To prevent any acute carryover effects from the last training bout, experimental procedures were delayed for 48 h after the last training session. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body mass) immediately (i.e., 10–15 min) before all experimental procedures. Surgical removal of muscle tissues for incubation and analyses required ~10 min, after which the rat was euthanized by an intracardiac injection of pentobarbital sodium. All procedures were approved by the University of Guelph Animal Ethics Committee.

Blood and tissue sampling. Blood was collected at the completion of treatments (4 wk) via cardiac puncture after the excision of the soleus (SOL, slow oxidative), red gastrocnemius (RG, fast oxidative glycolytic) and white gastrocnemius (WG, fast glycolytic) muscles. Gastrocnemius muscles were frozen immediately and stored in liquid nitrogen until further analysis, and the SOL muscle was prepared for incubation (see Muscle Incubations). All samples were taken following an overnight fast. Blood was transferred to a microcentrifuge tube where it was allowed to clot (25 min) before being centrifuged (12,000 g for 2 min) and the serum collected. Serum leptin and insulin were assayed in duplicate using RIA specific kits (Linco, St. Charles, MO). Nonesterified fatty acids were assayed using a Wako NEFA kit (Wako Chemical, Richmond, VA) and measured on the spectrophotometer (Beckman DU-70, Mississauga, ON, Canada) at 550 nm. Serum glucose was determined fluorimetrically (PerkinElmer LS50, Wellesley, MA) (4). RG and WG muscle TAG and glycogen contents were determined on freeze-dried samples, which were dissected free of all visible connective tissue and blood.

Quantification of SOCS3 and GAP T mRNA via real-time PCR. Total RNA was prepared from ~30 mg of rat tissues using TRIzol (Invitrogen, Mount Waverley, VIC, Australia) and purified according to the RNeasy cleanup protocol (Qiagen, Alameda, CA). Double-stranded cDNA was synthesized from 20 µg of total RNA according to the Affymetrix protocol for cDNA synthesis (GeneChip Xpression Analysis Technical Manual), and cDNA was purified using Eppendorf Phase Lock Gels. Real-time PCR reactions were set up using the QuantiTec SYBR-Green PCR kit (Qiagen) and were cycled according to Qiagen’s recommendations in a Roche LightCycler. Primer sequences designed to amplify transcripts of the housekeeping gene porphobilinogen deaminase (PBGD) and SOCS3 were as follows: PBGD (forward) 5′-CTGGGTGTGCTACTCTCTGA-3′ and (reverse) 5′-CAACAGATCACACAGGGTTT-3′; SOCS3 (forward) 5′-TGAGCGTCAAGACCCAGTCG-3′ and (reverse) 5′-CAGAGCAGGGGAAC-3′.

SOCS3 expression levels were expressed as ratios of SOCS3 detection relative to PBGD detection for each sample. Primer sequence and primer concentration were optimized for each gene using both standard curves and dissociation curves. The expression levels of SOCS3 were normalized relative to β-actin and GAP T expression under control and experimental conditions. Primer sequence information and primer concentration are available from the authors upon request.

Table 1. Final body mass and average daily caloric intake after 4 wk of treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body mass, g</th>
<th>Food intake, kcal/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>246.3 ± 7.3</td>
<td>53.8 ± 0.92</td>
</tr>
<tr>
<td>CT</td>
<td>233.9 ± 5.4</td>
<td>53.8 ± 0.92</td>
</tr>
<tr>
<td>FS</td>
<td>256.2 ± 5.8</td>
<td>51.3 ± 0.65</td>
</tr>
<tr>
<td>FT</td>
<td>236.0 ± 3.6</td>
<td>51.3 ± 0.65</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 animals per group. CS, control diet sedentary; CT, control diet trained; FS, fat diet sedentary; FT, fat diet trained.
quences designed to amplify transcripts of the housekeeping gene ribosomal protein L32 and GAPT were as follows: L32 (forward) 5'-CAGGGTTCTGAGGAGTCAAGGG-3' and (reverse) 5'-CTTAGAGGACAGGTGTGAGCAATC-3'; GAPT (forward) 5'-GGCGTATTACGAAATGCTGC-3' and (reverse) 5'-GCTGTCCTCAAAGGAGGGG-3'. GAPT expression levels were expressed relative to L32 detection for each sample.

Muscle incubations. Briefly, SOL muscles were carefully dissected into two longitudinal strips from tendon to tendon by means of a 27-gauge needle and placed in a 20-ml glass scintillation vial containing 3 ml of warmed (30°C), pregassed (95% O2 -5% CO2, pH 7.4) Krebs-Henseleit buffer containing 4% FA-free BSA (ICN Biomedicals, Mississauga, ON, Canada), 2 mM pyruvate, and 0.5 mM palmitate. This was the base buffer used in all experiments.

After a 20-min equilibration period, SOL strips were transferred to vials containing the base buffer plus 2 μCi of [1-14C]palmitate (Amersham Life Sciences, Oakville, ON, Canada) for 90 min. Vials contained either base buffer alone (control) or buffer plus leptin (10,000 ng/ml), a dosage previously demonstrated to maximally stimulate FA metabolism. Palmitate oxidation and incorporation into intracellular lipids (TAG, DAG, phospholipid (PL)) were monitored by 14CO2 production and [1-14C]palmitate incorporation using procedures described previously (12, 44).

After the incubation, muscles were homogenized in 5.0 ml of ice-cold 1:1 chloroform-methanol (vol/vol). Samples were then centrifuged at 2,000 g 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 before to separate the aqueous and lipophilic phases. One milliliter of the aqueous phase was quantified by liquid scintillation counting.

The quantity of palmitate esterified was calculated from the specific activity of labeled palmitate injected into each muscle, assuming 10 min of equilibration in the incubation medium. The specific activity of labeled palmitate was determined from a sample that was not incubated, but was kept at 30°C for the same time period as the incubation.

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., radioiodinated palmitate in dpm/total palmitate in nmol). All data are reported as means ± SE. Results were analyzed using analysis of variance (ANOVA) procedures, and a Tukey’s post hoc test was used to test significant differences revealed by the ANOVA. Significance was accepted at P ≤ 0.05.

RESULTS

Serum fatty acids, glucose, leptin, and insulin. FS rats had elevated serum glucose and leptin levels (Table 2). Endurance training in FT rats restored serum glucose and leptin to the levels of animals (CT) fed the control diet. Serum FA and insulin were unaltered with either treatment. Intramuscular TAG and glycogen contents. Endurance training was generally without effect on intramuscular TAG content (Table 3), although a significant effect was observed in WG when the dietary conditions were pooled (P < 0.05). Dietary treatment did not alter intramuscular TAG levels. Muscle glycogen content was also unaltered with diet or training in RG and WG (Table 3).

Metabolic responses to high-fat diet, endurance training, and leptin. Under basal conditions (i.e., absence of leptin) the combined effect of high-fat feeding and endurance training (FT) led to increased rates of total FA uptake (TAG + DAG + PL + oxidation) relative to CS (+49 ± 17%, P < 0.05; Fig. 1). TAG eстерification was also elevated in FT relative to CS (+40 ± 10%, P < 0.05; Fig. 2).

Leptin reduced TAG eстерification (CS − 17 ± 7%, CT − 24 ± 8%, P < 0.05; Fig. 2). This reduction in TAG eстерification was eliminated in rats fed a high-fat diet but restored with endurance training (− 19 ± 9%, P < 0.05). All

Table 2. Concentrations of serum substrates and hormones

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CS</th>
<th>CT</th>
<th>FS</th>
<th>FT</th>
</tr>
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<tbody>
<tr>
<td>FA, mmol</td>
<td>1.02 ± 0.10</td>
<td>0.82 ± 0.10</td>
<td>0.76 ± 0.06</td>
<td>0.82 ± 0.08</td>
</tr>
<tr>
<td>Glucose, mmol</td>
<td>4.85 ± 0.17</td>
<td>4.78 ± 0.24</td>
<td>5.62 ± 0.13†</td>
<td>5.14 ± 0.16</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>4.64 ± 1.09</td>
<td>2.81 ± 0.68</td>
<td>11.76 ± 2.51§</td>
<td>5.12 ± 1.24</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.10 ± 0.26</td>
<td>1.77 ± 0.30</td>
<td>1.71 ± 0.31</td>
<td>1.76 ± 0.34</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 animals per group. FA, fatty acid.

†Significantly different from CS, P = 0.06; †from CT, P < 0.05; §from all others, P < 0.05.
treatments were without effect on PL or DAG esterification (data not shown).

Leptin stimulated palmitate oxidation in both CS and CT (CS = 32 ± 15%, CT = 30 ± 17%, P < 0.05; Fig. 3). Leptin did not stimulate palmitate oxidation in animals fed a high-fat diet (FS). However, when endurance training was superimposed on the high-fat diet, palmitate oxidation in SOL was increased (+18 ± 13%, P = 0.0025) in the presence of leptin.

The combined effect of reduced esterification and increased oxidation with leptin in animals fed the control diet is demonstrated in the reduced esterification-to-oxidation ratio (CS = 19 ± 13%, CT = 29 ± 10%, P < 0.001; Fig. 4). This effect was eliminated in FS animals but was restored with endurance training (FT, = 20 ± 7%, P = 0.05).

SOCS3 and GPAT mRNA. High-fat feeding increased SOCS3 mRNA expression as determined by real-time PCR irrespective of training status (Fig. 5). GPAT mRNA was unaltered with high-fat feeding or endurance training (data not shown).

DISCUSSION

The novel findings of this study are that 1) the interaction of high-fat feeding and endurance training results in an increased total FA uptake in resting skeletal muscle, whereas these conditions independently do not; 2) high-fat feeding, independently of the development of obesity, induces leptin resistance in muscle while simultaneously stimulating the expression of SOCS3 (a potent inhibitor of leptin signaling) mRNA; 3) high-fat diet-induced skeletal muscle leptin resistance is partially reversed with endurance training, as demonstrated by the restoration of leptin’s ability to suppress TAG esterification; and 4) the partial restoration of leptin sensitivity is not accompanied by a decrease in SOCS3 mRNA expression, indicating the likely involvement of other factors in the regulation of leptin signaling.

Leptin resistance and SOCS3. Leptin administration in ob/ob mice has been demonstrated to cause a rapid reversal of obesity (3, 16). However, the relevance of this genetic model to...
the treatment of human obesity appears to be minimal. In contrast to leptin-deficient ob/ob mice, high levels of circulating leptin characterize nearly all cases of human obesity, although there are exceptions (10, 38, 39). Despite the presence of elevated circulating leptin concentrations, skeletal muscle TAG stores are elevated in the obese, and FA oxidation is decreased in vivo (18, 22). Several studies have shown that high-fat feeding in rodents leads to an increase in plasma leptin levels (1, 30), which are unable to prevent the development of obesity in these animals. In recent years, several studies have directly demonstrated impaired transport of leptin across the blood–brain barrier (9, 47) as well as the presence of leptin resistance in peripheral tissues such as skeletal muscle, liver, and white adipose tissue (44, 48–50). Furthermore, although recombinant leptin administration has pronounced effects in correcting insulin resistance in leptin-deficient, lipodystrophic humans (42), the physiological effects including weight loss appear to be minimal in the obese who are not leptin deficient, supporting the hypothesis that leptin resistance also exists in obese humans (19).

Because of the critical role of leptin in regulating skeletal muscle lipid metabolism and the development of leptin resistance in high-fat fed rats, we examined the expression of SOCS3 mRNA in skeletal muscle by using this model. SOCS3 expression is increased in hypothalamus and white adipocytes of leptin-resistant rodents (6, 49, 50); however, the role of SOCS3 in the development of leptin resistance in other peripheral tissues such as muscle has not been examined. Indeed, we are unaware of any reports to date that have measured the SOCS3 protein in skeletal muscle. In this study, we demonstrate that SOCS3 mRNA expression is elevated to a similar degree following high-fat feeding, as observed previously in white adipose tissue of animals fed a high-fat diet (+350%; P < 0.05). However, unlike the study by Wang et al. (49), in which rats were made obese by the high-fat diet, our findings indicate that SOCS3 mRNA expression is elevated in the absence of changes in body mass. Therefore, the development of skeletal muscle leptin resistance and SOCS3 mRNA expression caused by the consumption of a high-fat diet per se may be an important factor in the etiology of obesity. However, it is imperative that future studies examine the expression of the SOCS3 protein in muscle and its relationship to leptin sensitivity.

**Endurance training.** Despite the physiological relevance of leptin resistance to many aspects of health, including the ability to maintain appropriate body mass, insulin sensitivity, and glucose homeostasis, as well as many other physiological processes regulated by leptin, we are unaware of any intervention study that has attempted to attenuate or reverse the development of leptin resistance in either humans or rodents. In this study, we have utilized an endurance training protocol previously shown to improve insulin sensitivity in rats consuming high-fat diets (23, 25) in an attempt to reverse or attenuate the leptin resistance developed in oxidative skeletal muscle subsequent to the consumption of a high-fat diet. Furthermore, the effect of intense aerobic training on leptin sensitivity in sedentary rodents fed a control (i.e., high-carbohydrate) diet has also been untested.

Previous studies in both rodents and humans have reported reductions in circulating leptin as a result of endurance training, and in most cases these changes have occurred independently of alterations in body mass, suggesting, indirectly, that leptin sensitivity was improved (20, 40, 41). The data from the present study indicate that serum leptin is significantly elevated in sedentary rats fed a high-fat diet but not in fat-fed rats that were aerobically trained, indirectly supporting the hypothesis that leptin sensitivity was improved. In this study, we assessed skeletal muscle leptin sensitivity directly by measuring changes in FA metabolism in response to leptin. Despite the reduction of serum leptin in endurance-trained animals fed a control diet (FC), there was no evidence of improved leptin sensitivity in isolated SOL strips relative to untrained rats. This suggests 1) that leptin’s ability to stimulate FA oxidation may already be maximized in control muscles and/or 2) that changes in circulating leptin levels may not reflect skeletal muscle’s sensitivity to leptin, as we have previously noted a dissociation of this relationship (44). Endurance training also blunted the high-fat diet-induced increase in serum glucose, a probable index of improved insulin sensitivity (not determined in this study), but had little effect on circulating FA and insulin. In general, training also did not alter muscle glycogen and TAG contents in either RG or WG muscle. Dietary intervention was also without effect on muscle glycogen or TAG content. Although it might be expected that a high-fat diet would result in an elevated TAG content, this was not the case. However, this is in agreement with the lack of dietary effect on labeled palmitate incorporation into the TAG pool, observed in both the present and our recent study (44). This lack of effect may be due to a dietary intervention that was too brief (only 4 wk) or to the fact that the caloric intake was matched in the control sedentary and high-fat-fed groups. Alternatively, it is also possible that a larger sample size is required to detect small but significant changes in basal TAG metabolism and content that occurs in a relatively short period.

Our major finding was that endurance training restored the ability of leptin to decrease TAG esterification in rats fed a high-fat diet. Thus, with the superimposition of training, muscle from fat-fed rats had a rate of TAG esterification similar to that of sedentary carbohydrate-fed rats. Surprisingly, the ability of leptin to stimulate FA oxidation was not restored in fat-fed rodents after aerobic training. However, it should be noted that the basal rate of FA oxidation in SOL strips from the fat-fed rodents was already elevated, as we have previously shown (44), and may have been maximal, given the low metabolic rate of the isolated strips, thus precluding any additional stimulation by leptin. Alternatively, it is possible that there was a relatively small stimulation of FA oxidation in this group that was not detectable with our methods. Total FA uptake (PL + DAG + TAG + oxidation) was significantly increased in both trained groups (i.e., regardless of diet), suggesting an increase in FA transporter abundance/activity in the sarcolemma. Increases in plasma FABPp following endurance training in humans (24) and in FAT/CD36 following chronic stimulation in rodents (8) have previously been shown. Interestingly, the increase in FA uptake in muscle from trained rodents appears to be directed primarily toward incorporation into TAG rather than oxidation. This is likely due to the nature of the muscle preparation (i.e., low metabolic rate) and the inability to further increase FA oxidation in response to the enhanced uptake.

In summary, we have confirmed our previous observations that a 4-wk high-fat diet results in an impairment of leptin’s ability to partition FA incorporated into the muscle toward
oxidation and away from TAG storage. Contrary to our expectation, the addition of an intense aerobic training component only partially restored the sensitivity of oxidative rodent muscle to leptin, i.e., reduced TAG storage, but not increased FA oxidation. However, it is entirely possible that our inability to detect an increase in FA oxidation with leptin following endurance training in the fat-fed rodents was due to limitations in our model, i.e., resting rates of FA oxidation that were already maximal. Interestingly, although the development of leptin resistance coincided with the increased expression of SOCS3, a potent inhibitor of leptin signaling, the partial restoration of leptin’s effects with training did not. This suggests that other factors are involved in the regulation of leptin signaling or that the measured SOCS3 mRNA levels are not representative of changes in the actual protein content.

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