Both pharmacological intervention (i.e., thiazolidinediones [TZDs]) and lifestyle modification (i.e., exercise training) are clinically effective treatments for improving whole-body insulin sensitivity. However, the mechanism(s) by which these therapies reverse lipid-induced insulin resistance in skeletal muscle is unclear. We determined the effects of 4 weeks of rosiglitazone treatment and exercise training and their combined actions (rosiglitazone treatment and exercise training) on lipid and glucose metabolism in high-fat–fed rats. High-fat feeding resulted in decreased muscle insulin sensitivity, which was associated with increased rates of palmitate uptake and the accumulation of the fatty acid metabolites ceramide and diacylglycerol. Impairments in lipid metabolism were accompanied by defects in the Akt/AS160 signaling pathway. Exercise training, but not rosiglitazone treatment, reversed these impairments, resulting in improved insulin-stimulated glucose transport and increased rates of fatty acid oxidation in skeletal muscle. The improvements to glucose and lipid metabolism observed with exercise training were associated with increased AMP-activated protein kinase α1 activity; increased expression of Akt1, peroxisome proliferator–activated receptor γ coactivator 1, and GLUT4; and a decrease in AS160 expression. In contrast, rosiglitazone treatment exacerbated lipid accumulation and decreased insulin-stimulated glucose transport in skeletal muscle. However, rosiglitazone, but not exercise training, increased adipose tissue GLUT4 and acetyl CoA carboxylase expression. Both exercise training and rosiglitazone decreased liver triacylglycerol content. Although both interventions can improve whole-body insulin sensitivity, our results show that they produce divergent effects on protein expression and triglyceride storage in different tissues. Accordingly, exercise training and rosiglitazone may act as complementary therapies for the treatment of insulin resistance. Diabetes 56:1856–1864, 2007

Both pharmacological intervention (i.e., thiazolidinediones [TZDs]) and lifestyle modification (i.e., exercise training) are clinically effective treatments for improving whole-body insulin sensitivity (1,2). Yet, the mechanism(s) by which these therapies reverse lipid-induced insulin resistance in skeletal muscle is unclear. It has been suggested that peroxisome proliferator–activated receptor (PPAR) agonists, such as rosiglitazone, improve skeletal muscle insulin sensitivity by preventing the toxic accumulation of lipids in this tissue (3,4). However, the sequestration of lipids from skeletal muscle after chronic rosiglitazone treatment has not been consistently observed (3–11). There is also evidence that mechanism(s) independent of changes in lipid status (i.e., skeletal muscle AMP-activated protein kinase [AMPK] activation) may be involved in the insulin-sensitizing actions of rosiglitazone (12,13). Exercise training also induces several adaptations that may promote glucose uptake and fatty acid oxidation in skeletal muscle, including increased mitochondrial biogenesis, improved insulin signal transduction, and elevated GLUT4 protein content (14,15).

The potential for rosiglitazone and exercise training to improve skeletal muscle insulin sensitivity by multiple mechanisms raises the possibility that, in combination, these treatments may produce additive effects. In support of this contention, Hevener et al. (16) demonstrated that the PPARγ agonist troglitazone and exercise training had additive effects on whole-body insulin sensitivity in obese Zucker rats. However, it is unclear whether these effects were attributable to improvements in skeletal muscle insulin sensitivity or other insulin-sensitive tissues (i.e., liver and adipose). Furthermore, it is not known whether the combined insulin-sensitizing effects of these treatments are associated with improvements in muscle lipid metabolism.

Accordingly, in the present investigation, we used a model of lipid-induced insulin resistance, the high-fat–fed rat, to examine the independent and interactive effects of rosiglitazone treatment and exercise training on insulin-stimulated glucose transport, fatty acid oxidation, and lipid accumulation in skeletal muscle. The effects of these treatments on key regulators of glucose and lipid metabolism in the liver and adipose were also examined. It was hypothesized that a combination of exercise training and rosiglitazone treatment would result in additive improvements in skeletal muscle insulin sensitivity and lipid metabolism compared with either treatment alone.
RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (~4 weeks of age) were given ad libitum access to a high-fat diet (58% kcal fat; Research Diets, New Brunswick, NJ) for 4 weeks to induce insulin resistance. During the subsequent 4-week experimental period, rats continued to eat a high-fat diet and were randomly allocated to one of the following groups (n = 24/group): high-fat control, high-fat exercise training, high-fat rosiglitazone treatment, or a combination of high-fat exercise training and high-fat rosiglitazone treatment. Exercise training consisted of treadmill running for 1 h/day, 5 days/week on a 15% incline, at a speed that was gradually increased during the 1st week of training to 32 m/min. High-fat rosiglitazone-treated rats received a diet containing 50 ppm rosiglitazone (GlaxoSmithKline, Stevenage, U.K.), which they consumed ad libitum at an average dose of 2.08 ± 0.06 mg · kg⁻¹ · day⁻¹. A fifth group of rats (chow-fed controls; n = 24) was fed a normal chow diet (17% kcal fat; Research Diets) for the duration of the study (8 weeks) and acted as a control for high-fat controls. After the experimental period, animals were fasted for 8–12 h before undergoing hindlimb perfusion for the measurement of basal 3-O-methylglucose transport (n = 5/treatment group), insulin-stimulated 3-O-methylglucose transport (n = 8/treatment group), or [¹⁴C]palmitate uptake and oxidation (n = 8/treatment group). Exercise training animals undertook their last training bout 36–48 h before hindlimb perfusion.

Hindlimb perfusions. Rats were anesthetized and surgically prepared for hindlimb perfusion as previously described (17). The basic perfusate medium contained 30% washed time-expired human erythrocytes (Ogden Medical Center, Ogden, UT) and was continuously gassed with a mixture of 95% O₂-5% CO₂ and warmed to 37°C.

3-O-methylglucose transport. Insulin-stimulated perfusions were performed in the presence of 500 μM insulin, whereas basal perfusate contained no insulin. Glucose transport was measured over an 8-min period using a perfusate that contained 8 mmol/l nonmetabolizable glucose analog (CytoChem, Boston, MA) and 2 mmol/l mannitol (60 μM [¹⁴C]mannitol · mmol⁻¹ · l⁻¹; PerkinElmer Life Sciences, Boston, MA) and was continuously gassed with a mixture of 95% O₂-5% CO₂ and warmed to 37°C. Rates of basal and insulin-stimulated skeletal muscle 3-O-methylglucose transport (17) and insulin receptor substrate 1 (IRS1)-associated phosphatidylinositol 3-kinase (PI 3-kinase) activity (18) were calculated as previously described.

[¹⁴C]palmitate uptake and oxidation rates. Rats were fasted for 8–12 h before being anesthetized and having their hindlimbs surgically prepared for perfusion. Before cannulation, unperfused tissue from the left hindlimb was excised and stored for later analysis of AMPK activity and phosphorylation. Skeletal muscle fatty acid metabolism was assessed as previously described (19) by perfusing hindlimbs (7.5 ml/min) with a recirculated perfusate containing 4% FA-free BSA, 500 μM/l albumin-bound palmitate, and 5 μCi albumin-bound [¹¹C]palmitate (PerkinElmer) for 60 min. Arterial and venous samples for the analysis of [¹⁴C]CO₂ were taken immediately after a 20-min equilibration period and at 60 min. The liberation and collection of [¹⁴C]CO₂ from perfusate samples was performed by injecting 2 ml anaerobically collected perfusate into a sealed flask containing an equal volume of 1 mol/l acetic acid. The released [¹⁴C]CO₂ was trapped by an insert containing a strip of filter paper saturated with 500 μl benzozonium hydroxide and quantified using liquid scintillation counting.

AMPK activity and AMPK subunit protein expression. AMPK activity, pThr172 phosphorylation, and total protein content were analyzed as previously described (20). Activities were calculated as picomoles of phosphate incorporated into the SAMS peptide per minute, per milligram of protein subjected to immunoprecipitation. Acetyl CoA carboxylase (ACC) was affinity purified and quantified using post-AMPK immunoprecipitation supernatants as previously described (20).

RESULTS

Body mass was increased by high-fat feeding and rosiglitazone treatment but was decreased by exercise training (P < 0.05, Table 1). Changes in body mass were mirrored by changes in epididymal fat pad mass (Table 1). Serum adiponectin concentration was elevated in high-fat rosiglitazone (P = 0.03 vs. high-fat controls) and decreased in high-fat exercise training (P = 0.04 vs. high-fat controls). Serum FFA concentration was decreased in high-fat exercise training and in high-fat rosiglitazone treatment and exercise training (P < 0.02 vs. high-fat control; Table 1).

Glucose metabolism. Insulin-stimulated glucose transport in the red quadriceps muscle of high-fat controls (high-fat controls) was reduced by ~37% (P = 0.01; Fig. 1A) compared with chow-fed controls (chow-fed controls) and was impaired a further ~32% (P = 0.05) in high-fat rosiglitazone. Exercise training increased insulin-stimulated glucose transport in fat-fed and rosiglitazone-treated animals (P < 0.05). Insulin-stimulated IRS1-associated PI 3-kinase activity in high-fat controls was reduced by 39% compared with chow-fed controls (P = 0.05; Fig. 1B). Exercise training increased insulin-stimulated PI 3-kinase activity in fat-fed (P = 0.01) but not in rosiglitazone-treated animals (high-fat exercise training vs. high-fat rosiglitazone treatment and exercise training, P < 0.01).

TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Chow-fed controls</th>
<th>High-fat controls</th>
<th>High-fat exercise training</th>
<th>High-fat rosiglitazone</th>
<th>High-fat exercise training and rosiglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>434 ± 6</td>
<td>464 ± 8*</td>
<td>417 ± 5†</td>
<td>470 ± 8‡</td>
<td>404 ± 7†§</td>
</tr>
<tr>
<td>Epididymal fat pad mass (g)</td>
<td>6.2 ± 0.3</td>
<td>10.7 ± 0.6*</td>
<td>6.8 ± 0.4†</td>
<td>10.3 ± 0.5‡</td>
<td>6.6 ± 0.3§</td>
</tr>
<tr>
<td>Serum glucose (mmol/l)</td>
<td>9.9 ± 0.9</td>
<td>9.8 ± 0.5</td>
<td>9.9 ± 0.8</td>
<td>9.3 ± 0.9</td>
<td>7.0 ± 1.0‡</td>
</tr>
<tr>
<td>Serum insulin (ng/ml)</td>
<td>1.23 ± 0.25</td>
<td>1.21 ± 0.24</td>
<td>0.84 ± 0.16</td>
<td>1.25 ± 0.21</td>
<td>0.88 ± 0.20</td>
</tr>
<tr>
<td>Serum adiponectin (ng/ml)</td>
<td>2.09 ± 0.30</td>
<td>3.46 ± 0.80</td>
<td>1.58 ± 0.26</td>
<td>5.83 ± 0.65‡</td>
<td>3.70 ± 0.5§</td>
</tr>
<tr>
<td>Serum FFA (mmol/l)</td>
<td>0.80 ± 0.16</td>
<td>0.60 ± 0.10</td>
<td>0.33 ± 0.05†</td>
<td>0.50 ± 0.05</td>
<td>0.29 ± 0.04‡</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 vs. chow-fed controls; †P < 0.05 vs. high-fat controls; ‡P < 0.05 vs. high-fat exercise training, §P < 0.05 vs. high-fat rosiglitazone.
Lipid metabolism.

high-fat controls). high-fat exercise training and high-fat rosiglitazone treatment and exercise training (P < 0.05 vs. high-fat control; Fig. 2F). No changes in the protein content of FABPpm were observed (data not shown). Although [14C]palmitate uptake was not increased in high-fat exercise training (Fig. 2D), a twofold increase in 14CO2 production was observed (P = 0.03 vs. high-fat control; Fig. 2E), suggesting that increased lipid oxidation may be a mechanism responsible for normalized lipid levels in high-fat rosiglitazone treatment and exercise training. Increased lipid oxidation with exercise training was associated with ~30% increase in the expression of PGC-1 (Fig. 3D) in high-fat exercise training (P = 0.03 vs. high-fat controls) and in high-fat rosiglitazone treatment and exercise training (P = 0.07 vs. high-fat controls).

AMPK activity. AMPKα1 activity was unchanged by high-fat feeding but increased ~30% in high-fat exercise training (P = 0.01 vs. high-fat controls) and ~37% in high-fat rosiglitazone treatment and exercise training (P = 0.006 vs. high-fat controls; Fig. 3A). AMPKα2 activity was increased ~36% by high-fat feeding (P = 0.05, high-fat vs. chow-fed controls) but was not further altered by exercise training or rosiglitazone (Fig. 3B). Changes in both AMPKα1 and α2 activities were associated with similar changes in the total protein levels of these isoforms (P < 0.05, Fig. 3C). Phosphorylation of AMPKα1 at T172 was similar between high-fat controls and high-fat rosiglitazone but tended to increase in high-fat exercise training and in high-fat rosiglitazone treatment and exercise training (P = 0.1 vs. high-fat controls). High-fat–induced increases in activity and protein levels in the AMPKα2 isoform were associated with increased phosphorylation of the enzyme at T172 (chow-fed vs. high-fat controls, P = 0.01). We also examined the protein levels and phosphorylation status of the AMPK substrate, ACC. Although no changes in the total protein level of ACC were observed (Fig. 3C) there was a significant increase in the level of phosphorylation of the enzyme at S218 with high-fat feeding (chow-fed vs. high-fat controls, P = 0.04).

Akt, AS160, and GLUT4. Compared with chow-fed controls, total Akt protein was reduced ~25% by high-fat feeding (high-fat control; P = 0.02) and was restored by exercise training (high-fat controls vs. high-fat exercise training; P = 0.007; Fig. 4A). The increase in total Akt was entirely attributable to an increase in the Akt1 isoform (Fig. 4C), which was elevated ~50% by exercise training (high-fat controls vs. high-fat exercise training; P = 0.004). Rosiglitazone did not change Akt1 protein content and appeared to blunt the exercise training–induced increase in Akt1 (high-fat exercise training vs. high-fat rosiglitazone treatment and exercise training; P = 0.04). No changes in the Akt2 isoform (Fig. 4D) were observed after either treatment. Phosphorylation of total Akt at the Ser473 residue (Fig. 4B) was also altered by exercise training, with a 50% decrease in high-fat exercise training (P = 0.01 vs. high-fat controls). AS160 protein was elevated ~15% in high-fat (chow-fed vs. high-fat controls; P = 0.004) and was normalized in high-fat exercise training (P = 0.04) and in high-fat rosiglitazone treatment and exercise training (P = 0.03; Fig. 4E). GLUT4 protein content was decreased ~20% in high-fat controls (P = 0.007) but was increased ~50 and ~40% in high-fat exercise training and in high-fat rosiglitazone treatment and exercise training, respectively (P < 0.001; Fig. 4F).
Adipose tissue analysis. GLUT4 content in adipose tissue was reduced by ∼20% in high-fat controls compared with chow-fed controls (P = 0.01) and tended to be impaired a further ∼20% by exercise training (high-fat exercise training, P = 0.07; Fig. 5A). Rosiglitazone increased adipose tissue GLUT4 content in both fat-fed (high-fat controls vs. high-fat rosiglitazone, P = 0.04) and exercise-trained (high-fat exercise training vs. high-fat rosiglitazone treatment and exercise training, P = 0.007) animals. Adipose tissue PGC-1 protein content was decreased by exercise training (high-fat controls vs. high-fat exercise training, P < 0.05; Fig. 5F) and increased by rosiglitazone treatment (P = 0.05). PEPCK content in adipose tissue was increased by high-fat feeding (chow-fed vs. high-fat controls, P < 0.05; Fig. 5F) and was elevated further by rosiglitazone (P < 0.01). AMPKα1 activity and protein content were elevated in high-fat compared with lean controls (P < 0.05; Fig. 5D and E). Rosiglitazone treatment tended to decrease AMPKα1 activity (P = 0.1) and protein content (P < 0.03) while increasing the expression of ACC protein in adipose tissue (P < 0.05; Fig. 5F).

Liver analysis. Liver TAG was increased 36% by high-fat feeding (P = 0.05), but was reduced in all other treatment groups (P < 0.001 vs. high-fat controls; Fig. 6A). Exercise training resulted in a small (∼13)% but significant increase in liver FAT/CD36 expression (P = 0.03; Fig. 6B), whereas rosiglitazone treatment decreased the expression of this fatty acid transporter (P < 0.02 vs. high-fat exercise training). Liver PGC-1 expression was increased (P = 0.01 vs. chow-fed controls; Fig. 6C) and PEPCK expression was decreased (P = 0.04 vs. chow-fed controls; Fig. 6D) by high-fat feeding, with little effect of exercise training or rosiglitazone.

DISCUSSION

It is well-established that chronic rosiglitazone treatment improves whole-body insulin sensitivity (23). Less clear, however, is whether this enhanced insulin action is directly attributable to the effects of rosiglitazone on skeletal muscle or other insulin-sensitive tissues. In the present study, we observed that exercise training, but not rosiglitazone treatment, reversed skeletal muscle insulin resistance associated with a high-fat diet. Contrary to our hypothesis, rosiglitazone treatment decreased insulin-stimulated glucose transport (P = 0.05, Fig. 1A) in the perfused hindlimb compared with high-fat controls.

Reduced skeletal muscle insulin sensitivity with rosiglitazone treatment seems a paradoxical finding given the ability of the drug to improve whole-body insulin sensitivity (23). Other investigations have demonstrated enhanced skeletal muscle and whole-body insulin sensitivity after short-term (4 days [7] or 2 weeks [24]) rosiglitazone treatment in high-fat–fed rats. We are not the first, however, to report a failure of TZDs to improve insulin resistance induced by high-fat feeding in rodents (25,26).

Our observation of increased muscle FAT/CD36 expression and lipid storage provides a potential mechanism for an absence of rosiglitazone-induced muscle sensitization. It is possible that the longer duration of high-fat feeding (8 weeks) and rosiglitazone treatment (4 weeks) used in the present study provided sufficient time to allow for the accumulation of muscle lipids, resulting from increased CD36 expression. A time-course experiment would be necessary to determine whether muscle insulin sensitization observed after shorter term (<2 weeks) TZD treatment is abolished as treatment duration increases in fat-fed animals.

The "lipid steal hypothesis" of TZD action postulates...
FIG. 3. Skeletal muscle AMPK activity and protein expression. AMPKα1 (A) and AMPKα2 (B) activities (pmol·min⁻¹·mg⁻¹) were measured using the SAMS peptide assay in protein isolated by immunoprecipitation of muscle lysates. Relative levels of isoform-specific AMPK T172 phosphorylation and total protein, ACC S218 phosphorylation and total protein (C), and PGC-1 total protein (D) were quantified using Western blot analysis and densitometry. SDs between groups (P < 0.05) are indicated by the P values listed on the figure; n = 7–8/group.

FIG. 4. AS160, GLUT4, and isoform-specific skeletal muscle Akt content. Relative protein levels of total Akt 1/2 (A), Akt phosphorylation at Ser473 (B), isoform-specific Akt1 (C) and Akt2 (D), and total AS160 (E) and GLUT4 (F) were quantified using Western blot analysis and densitometry. SDs between groups (P < 0.05) are indicated by the P values listed on the figure; n = 7–8/group.
that by increasing fatty acid storage in adipose tissue, TZDs enhance insulin sensitivity by reducing the toxic accumulation of lipids in other insulin-sensitive tissues such as skeletal muscle and liver (5). However, there is mounting evidence that rosiglitazone acts to improve insulin sensitivity in the absence of decreased muscle lipid storage in humans (9,27) and animal models of insulin resistance (7,10,12,28), suggesting that lipid steal from muscle may not be necessary for rosiglitazone-induced insulin sensitization. Although some investigations have demonstrated that lipid storage is decreased in muscle after rosiglitazone treatment (3,4,24), rosiglitazone-induced reductions of liver lipid accumulation have been more consistently observed (5,7,9,29). In the present study, 4-week rosiglitazone treatment resulted in a 20% increase in muscle TAG content (Fig. 3).

An alternative mechanism by which rosiglitazone may improve whole-body insulin sensitivity is by enhancing skeletal muscle glucose uptake via activation of AMPK in this tissue (13). In this regard, we have previously demonstrated that chronic rosiglitazone restores AMPK activation in the skeletal muscle of fa/ fa Zucker rats, a model that displays reduced AMPK activation as a result of deficient leptin signaling (20). In contrast, high-fat feeding in the present study resulted in a ~36% increase in AMPKα2 activity (Fig. 3B), which was not further augmented by rosiglitazone treatment. Thus, when compared with models lacking intact leptin signaling (i.e., ob/ob mouse, fa/ fa rat) (20,31,32), the absence of AMPK deficiency in the present model of diet-induced insulin resistance provides a likely mechanism for the absence of a rosiglitazone-induced improvements in AMPK activation and muscle glucose transport. Our in vivo finding of increased AMPKα2 activity with high-fat feeding is in agreement with previous work demonstrating that acutely increasing fatty acid availability stimulates AMPK activity, ACC phosphorylation, and palmitate oxidation in L6 myotubes (33). In contrast, AMPKα1 activity was not affected by diet but was increased by exercise training, which was associated with improved insulin-stimulated glucose transport and glycogen storage. These observations are consistent with other reports of upregulation of AMPKα1 after exercise training in humans with type 2 diabetes (34) and animal models of insulin resistance (35). Our results suggest distinct roles for the AMPKα subunit isoforms in skeletal muscle, with AMPKα2 activity being responsive to increased fatty acid availability and AMPKα1 activity being linked to exercise training–induced changes in glycogen storage and insulin sensitivity.

FIG. 5. Adipose tissue protein expression and AMPK activity. Relative levels of GLUT4 (A), PGC-1 (B), and PEPCK (C) protein were quantified in 30-μg aliquots of adipose tissue lysates using Western blotting and densitometry. AMPKα1 (D) activity (pmol · min⁻¹ · mg⁻¹) was measured using the SAMS peptide assay in protein isolated by immunoprecipitation of adipose lysates. Relative levels of AMPKα1 T172 phosphorylation and total protein (E) and ACC S218 phosphorylation and total protein (F) were quantified using Western blot analysis and densitometry. SDs between groups (P < 0.05) are indicated by the P values listed on the figure; n = 8/group.
In addition to increasing AMPK activity, exercise training may also improve glucose uptake via enhancement of the classic insulin signaling pathway. In the present study, exercise training increased IRS1-associated PI 3-kinase activity and normalized impairments to total protein levels in the Akt/AS160/GLUT4 signaling pathway caused by high-fat feeding. To our knowledge, the present investigation is the first to demonstrate changes in AS160 expression due to chronic high-fat feeding and exercise training. Larance et al. (21) established that decreasing AS160 expression by short hairpin RNA leads to increased plasma membrane GLUT4 content. However, it is unclear what the physiological effects of altered AS160 expression are in vivo. We also observed an increase in the expression of the Akt1 isoform (Fig. 4C) after exercise training. Akt1 has recently been implicated in the regulation of fatty acid uptake in skeletal muscle using isoform-specific siRNA-based gene silencing (36); however, more investigation into the specific functions of Akt1 and AS160 in exercise training–induced adaptations is needed.

Although exercise did enhance PI 3-kinase activation, we observed a seemingly paradoxical decrease in Akt phosphorylation at Ser473 in exercise-trained animals (Fig. 4B). However, discrepancies between Akt activation and upstream insulin signaling have been reported in several different models of insulin resistance (37–39). In previous investigations, exercise training resulted in increased (40) or unchanged (41) insulin-stimulated Akt Ser473 phosphorylation, indicating that Akt activation is not necessary for exercise-induced insulin sensitization. Recent evidence suggests that AMPK activation results in the dephosphorylation of Akt at Ser473 (42). Decreased Akt Ser473 phosphorylation in the present investigation was associated with ~30% increases in AMPK activity with both exercise training and combined therapy (Fig. 3A), providing a potential mechanism for exercise-induced reductions in Akt activation.

In contrast to exercise, we saw no change in IRS1-associated PI 3-kinase activity (Fig. 1B) or the total protein levels of Akt, AS160, or GLUT4 (Fig. 4) after 4 weeks of rosiglitazone treatment. These findings are in agreement with those of Karlsson et al. (43) who observed no enhancement of the IRS1/PI 3-kinase/Akt/AS160 signaling pathway after 26 weeks of rosiglitazone treatment (8 mg/day) in the skeletal muscle of patients with newly diagnosed type 2 diabetes. However, despite no apparent improvement in muscle insulin signaling, insulin-stimulated muscle glucose uptake was increased in these patients (43). In contrast, others have observed improvements in insulin signaling in patients with type 2 diabetes after rosiglitazone treatment (44,45). Thus, it is not clear whether rosiglitazone-induced insulin sensitization in humans is attributable to improved insulin signaling in muscle. Our results suggest that impairments to muscle insulin signal transduction that result from high-fat feeding are reversed by exercise training, but not rosiglitazone treatment. This contention is supported by recent clinical evidence demonstrating that lipid-induced insulin resistance in obese individuals is not prevented by rosiglitazone treatment (46).

In contrast to its beneficial effects on glucose and lipid
metabolism in skeletal muscle, exercise training appears to have an inhibitory role on these metabolic pathways in adipose tissue. Exercise training reduced adipose tissue GLUT4 (Fig. 5A), PGC-1 (Fig. 5B), and ACC (Fig. 5F) protein content. A combination of these adaptations would likely reduce glucose uptake and fat storage in adipose tissue, presumably functioning to spare glucose and lipid substrates for use in active skeletal muscle. Notably, the negative effects of exercise on key enzymes involved in glucose uptake and lipid storage in adipose tissue were reversed by rosiglitazone treatment. In adipose tissue, rosiglitazone increased GLUT4 (Fig. 5A) and PGC-1 protein content (Fig. 5B), both of which are associated with insulin sensitivity in adipocytes (47). Rosiglitazone also increased the expression of key proteins involved in glyceroenogenesis, lipogenesis, and lipid storage in adipocytes, such as PEPCK (Fig. 5C) (48) and ACC (Fig. 5F). We also observed a rosiglitazone-induced decrease in adipose tissue AMPKα1 expression and a tendency (P = 0.1) for reduced AMPK activation. Given that AMPK activation (5-aminoimidazole-4-carboxamide ribonucleoside) with AICAR has been recently shown to inhibit glucose uptake and lipogenesis in adipocytes (49), our results are consistent with increased adipose tissue glucose uptake and lipid storage. In support of this contention, Virtanen et al. (50) demonstrated that enhanced glucose uptake into adipose tissue makes a significant contribution to rosiglitazone-induced insulin sensitization in patients with newly diagnosed type 2 diabetes. Our observations of increased adipose tissue mass combined with elevated adipose GLUT4 content and reduced AMPK activation provide a potential mechanism for increased adipose tissue glucose uptake after rosiglitazone treatment. Thus, it is possible that the increased adipose tissue mass commonly observed after TZD treatment (23) may provide a “glucose sink” that contributes to the ability of rosiglitazone to improve peripheral glucose disposal.

Our observation that lipid accumulation was reduced in the liver of rosiglitazone-treated animals (Fig. 6A) raises the possibility that this organ also acts as a potential target for the insulin-sensitizing actions of the drug. In support of this contention, Castaldelli et al. (51) have recently reported that rosiglitazone treatment (8 mg/day for 12 weeks) decreased gluconeogenesis in patients with type 2 diabetes. Thus, it is probable that exercise-induced improvements in skeletal muscle insulin sensitivity, combined with the potential for rosiglitazone to improve adipose tissue glucose uptake, lipid storage, and hepatic insulin action, would produce additive improvements to whole-body insulin sensitivity as previously reported (16).

In conclusion, we provide novel data to demonstrate that exercise training, but not rosiglitazone treatment reverses skeletal muscle insulin resistance induced by lipid oversupply. Conversely, rosiglitazone, but not exercise training, increases the expression of proteins that regulate adipose tissue glucose transport and fatty acid storage. Although both interventions can improve whole-body insulin sensitivity, our results show that they produce divergent effects on protein expression and triglyceride storage in different tissues. Accordingly, exercise training and rosiglitazone may act as complementary therapies for the treatment of insulin resistance.

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