Differential Effects of Rosiglitazone on Skeletal Muscle and Liver Insulin Resistance in A-ZIP/F-1 Fatless Mice

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To determine the role of adipocytes and the tissue-specific nature in the insulin sensitizing action of rosiglitazone, we examined the effects of 3 weeks of rosiglitazone treatment on insulin signaling and action during hyperinsulinemic-euglycemic clamps in awake A-ZIP/F-1 (fatless), fat-transplanted fatless, and wild-type littermate mice. We found that 53 and 66% decreases in insulin-stimulated glucose uptake and insulin receptor substrate (IRS)-1–associated phosphatidylinositol (PI) 3-kinase activity in skeletal muscle of fatless mice were normalized after rosiglitazone treatment. These effects of rosiglitazone treatment were associated with 50% decreases in triglyceride and fatty acyl-CoA contents in the skeletal muscle of rosiglitazone-treated fatless mice. In contrast, rosiglitazone treatment exacerbated hepatic insulin resistance in the fatless mice and did not affect already reduced IRS-2–associated PI 3-kinase activity in liver. The worsening of insulin action in liver was associated with 30% increases in triglyceride and fatty acyl-CoA contents in the liver of rosiglitazone-treated fatless mice. In conclusion, these data support the hypothesis that rosiglitazone treatment enhanced insulin action in skeletal muscle mostly by its ability to repartition fat away from skeletal muscle. *Diabetes* 52:1311–1318, 2003

Thiazolidinediones (TZDs) are antidiabetic agents that improve glucose tolerance in patients with type 2 diabetes mostly through enhancing insulin sensitivity in skeletal muscle (1–6). Despite its widespread use, the mechanism by which TZDs increase insulin sensitivity in muscle is much debated. Because TZDs are high-affinity ligands for the transcription factor peroxisome proliferator–activated receptor-γ (PPAR-γ), which is expressed at high levels in adipose tissue (7,8), some investigators have hypothesized that the insulin-sensitizing action of TZDs may involve activation of PPAR-γ in adipocytes resulting in adipocyte differentiation/hyperplasia and partitioning of fat away from skeletal muscle and into adipocytes (9–14). In contrast, others have argued that TZDs may directly affect skeletal muscle insulin sensitivity by binding to PPAR-γ present in this tissue (15) and altering expression of genes involved in fat and glucose metabolism (8,16–18). Recently, Burant et al. (19) demonstrated that troglitazone altered glucose metabolism in ap2/DTA mice that partially lack white and brown adipose tissues and suggested that the insulin-sensitizing action of TZDs may be independent of adipose tissue, therefore supporting the latter hypothesis. In contrast, Chao et al. (20) recently demonstrated that rosiglitazone failed to improve hyperglycemia and glucose intolerance of A-ZIP/F-1 "fatless" mice, which virtually lack white adipose tissue, suggesting that adipose tissue is required for PPAR-γ's insulin sensitizing action. One possible explanation for these different results may be due to the differences in the severity of lipodystrophy, whereby A-ZIP/F-1 mice are more devoid of fat than ap2/DTA mice (19,21). Furthermore, neither of these previous studies examined the effect of rosiglitazone on insulin action in specific tissues (e.g., liver, skeletal muscle, adipocytes). Therefore, to determine the mechanism of TZDs' insulin-sensitizing action, we examined the effects of rosiglitazone treatment in A-ZIP/F-1, fat-transplanted A-ZIP/F-1, and wild-type littermate mice on insulin action and signaling in skeletal muscle, liver, and adipose tissue during hyperinsulinemic-euglycemic clamps and combined these measurements with liquid chromatography tandem mass spectrometry (LC/MS/MS) measurements of fatty acyl-CoA and ceramide content in these tissues.

RESEARCH DESIGN AND METHODS

Animals. Male wild-type (n = 10), fatless (A-ZIP/F-1; n = 13), and female fat-transplanted fatless (n = 19) mice were studied at 13 weeks of age (22–24 g of body weight) with or without 3 weeks of rosiglitazone treatment (3 mg/kg rosiglitazone mixed with an AIN-93G diet [20]). All of the mice were generated with FVB as the background strain. For the fat-transplantation, ~500 mg of parametrial fat from wild-type littermates was transplanted into dorsal subcutaneous tissue of fatless mice at 5 weeks of age (22). Fatless mice received a sham operation at the same age. Animals were housed under controlled temperature (23°C) and lighting (12-h light; 0600–1800, 12-h dark; 1800–0600) with free access to water and mouse diet. All procedures were

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2-DG-6-P, 2-[^{14}C]DG-6-phosphate; HGP, hepatic glucose production; IRS, insulin receptor substrate; LC/MS/MS, liquid chromatography tandem mass spectrometry; PI, phosphatidylinositol; PPAR-γ; peroxisome proliferator–activated receptor-γ; TNF-α, tumor necrosis factor-α; TZD, thiazolidinedione.

approved by the Yale University and National Institute of Diabetes and Digestive and Kidney Diseases Animal Care and Use Committees.

Surgery and animal handling. At least 4 days before hyperinsulinemic-euglycemic clamp experiments, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body wt) and xylazine (10 mg/kg body wt), and an indwelling catheter was inserted in the left internal jugular vein. The catheters were externalized through an incision in the skin flap behind the head, and the mice were returned to individual cages after the surgery. Mice had fully recuperated from the surgery before the in vivo experiments as reflected by their reaching preoperative weight. For conducting experiments in awake mice with minimal stress, a tail restraint method was used (23).

Hyperinsulinemic-euglycemic clamp to assess in vivo insulin action in awake mice. After an overnight fast, high-performance liquid chromatography–purified [3-^{3}H]glucose (0.05 μCi/min; NEN Life Science, Boston, MA) was infused during the 2-h basal period, and blood samples (~40 μl) were collected at the end to estimate the rate of basal hepatic glucose production. After the basal period, a 120-min hyperinsulinemic-euglycemic clamp was conducted with a primed-continuous infusion of human insulin (Humulin; Eli Lilly, Indianapolis, IN) at a rate of 15 pmol · kg^{-1} · min^{-1} to raise plasma insulin within a physiological range (~650 pmol/l). Blood samples (~20 μl) were collected at 20-min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose was infused at variable rates to maintain plasma glucose at basal concentrations. Insulin-stimulated whole-body glucose uptake and metabolism were estimated using a primed-continuous infusion of [3-^{3}H]glucose (10 μCi bolus, 0.1 μCi/min) throughout the clamps. All infusions were done using microdialysis pumps (CMA/Microdialysis; North Chelmsford, MA). For estimating insulin-stimulated glucose uptake in individual tissues, 2-deoxy-D-[1-^{14}C]glucose (2-[^{14}C]DG; NEN) was administered as a bolus (10 μCi) at 75 min after the start of clamps. Because 2-deoxyglucose is a glucose analog that is phosphorylated but not metabolized, insulin-stimulated glucose uptake in individual tissues can be estimated by determining the tissue content of 2-deoxyglucose-6-phosphate. Blood samples (20 μl) were taken at 80, 85, 90, 100, 110, and 120 min after the start of clamps for the determination of plasma [^{3}H]glucose, ^3H_2O, and 2-[^{14}C]DG concentrations. Additional blood samples (20 μl) were collected at the end of clamps for measurement of plasma insulin concentrations. At the end of clamp experiments, mice were anesthetized with sodium pentobarbital injection. Within 5 min, three muscles (gastrocnemius, tibialis anterior, and quadriceps) from both hindlimbs, epididymal white adipose tissue, intrascapular brown adipose tissue, liver, and heart were taken. Each tissue, once exposed, was dissected out within 2 s, frozen immediately using liquid N_2-cooled aluminum blocks, and stored at −70°C for later analysis.

In vivo glucose flux analysis and calculations. Plasma glucose concentration during the clamp study was analyzed using 10 μl of plasma by a glucose oxidase method on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA). Plasma insulin concentration was measured by radioimmunoassay using kits from Linco Research (St. Charles, MO). Tissue triglyceride concentrations were determined using Triglyceride Diagnostic Kit (Sigma Chemicals, St. Louis, MO) after tissue homogenization using chloroform/methanol. For the determination of plasma [3-^{3}H]glucose and 2-[^{14}C]DG concentrations, plasma was deproteinized with $ZnSO_4$ and $Ba(OH)_2$, dried to remove ^3H_2O, resuspended in water, and counted in scintillation fluid (Ultima Gold; Packard, Meridien, CT) on dual channels for separation of ^{3}H and ^{14}C. The plasma concentration of ^3H_2O was determined by the difference between ^{3}H counts without and with drying. The radioactivity of ^{3}H in tissue glycogen was determined by digesting tissue samples in KOH and precipitating glycogen with ethanol (23). For the determination of tissue 2-[^{14}C]DG-6-phosphate (2-DG-6-P) content, tissue samples were homogenized, and the supernatants were subjected to an ion-exchange column to separate 2-DG-6-P from 2-DG (23). Rates of basal hepatic glucose production and insulin-stimulated whole-body glucose uptake were determined as the ratio of the [^{3}H]glucose infusion rate (disintegrations per minute [dpm]/min) to the specific activity of plasma glucose (dpm/μmol) at the end of basal period and during the final 30 min of clamps, respectively. Hepatic glucose production during the hyperinsulinemic-euglycemic clamps was determined by subtracting the glucose infusion rate from the whole-body glucose uptake. Whole-body glycolysis was calculated from the rate of increase in plasma ^3H_2O concentration, determined by linear regression of the measurements at 80, 90, 100, 110, and 120 min. Whole-body glycogen and lipid synthesis were estimated by subtracting whole-body glycolysis from whole-body glucose uptake, assuming that glycolysis and glycogen/lipid synthesis account for the majority of insulin-stimulated glucose uptake (24). Glucose uptake in individual tissues was calculated from plasma 2-[^{14}C]DG profile, which was fitted with a double exponential curve using MLAB (Civilized Software, Bethesda, MD) and tissue 2-DG-6-P content (23). Skeletal muscle glycogen synthesis was calculated from ^{3}H incorporation to muscle glycogen (23). Skeletal muscle glycolysis was then estimated as the difference between muscle glucose uptake and glycogen synthesis.

Insulin signaling analysis. Tyrosine phosphorylation of insulin receptor substrate (IRS)-1/IRS-2 and IRS-1/IRS-2–associated phosphatidylinositol (PI) 3-kinase activity in skeletal muscle (gastrocnemius) and liver were measured by immunoprecipitating IRS-1/IRS-2 using antibodies to IRS-1/IRS-2 (provided by Dr. Morris White, Joslin Diabetes Center, Boston, MA) and assessing the incorporation of ^{32}P into PI to yield PI-3-monophosphate. Tyrosine phosphorylation of insulin receptor in skeletal muscle was measured using antiphosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY).

Mass spectrometry. For determining the tissue-specific concentrations of fatty acid–derived metabolites (i.e., fatty acyl-CoA and ceramide) using LC/MS/MS, skeletal muscle (quadriceps) and liver (~100 mg) were homogenized and prepared using a modified method of Bligh et al. (25). PE sciex API 3000 tandem mass spectrometer interfaced with TurboIonSpray ionization source was used for the analysis. The concentrations of long-chain fatty acyl-CoAs (C16, C16:1, C18, C18:1, and C18:2) were detected in negative electrospray mode, and C17 CoA ester was used as an internal standard. The doubly charged ions of these compounds were transmitted, and singly charged product ions were quantified in MRM mode. For the concentrations of ceramide, the transition pairs $[M+H-H_2O]^+/264.4$ (common product ion for all ceramides) were monitored in positive MRM mode for individual ceramide species, and C6 sphingosine was used as an internal standard.

Statistical analysis. Data are expressed as means ± SE. The statistical significance of the difference in mean values among wild-type, fatless, and fat-transplanted fatless mice with or without rosiglitazone treatment was evaluated using the Duncan's multiple range test.

RESULTS

Insulin action on glucose uptake and metabolism was examined during a 2-h hyperinsulinemic-euglycemic clamp in awake wild-type, fatless, and fat-transplanted fatless mice with or without 3 weeks of rosiglitazone treatment. Overnight fasted plasma glucose concentration was significantly increased in the fatless mice as compared with the wild-type mice (Table 1). After rosiglitazone treatment, plasma glucose concentration was significantly decreased when compared with the untreated fatless mice but remained elevated as compared with the wild-type mice (Table 1). In contrast, fat transplantation, with or without rosiglitazone treatment, normalized plasma glucose concentration in the fatless mice. Overnight fasted plasma insulin concentrations did not differ among the groups (Table 1). During the clamps, plasma insulin concentration was raised to ~650 pmol/l, whereas the plasma glucose concentration was maintained at ~7 mmol/l by a variable infusion of glucose in all groups (Table 1). The glucose infusion rate required to maintain euglycemia increased rapidly in the wild-type and rosiglitazone-treated wild-type mice and reached a steady state within 90 min. In contrast, there was a markedly blunted insulin response during the hyperinsulinemic-euglycemic clamp studies in the fatless and rosiglitazone-treated fatless mice, as reflected by a much lower steady-state glucose infusion rate in the fatless and rosiglitazone-treated fatless mice (78 ± 25 and 40 ± 12 μmol · kg^{-1} · min^{-1}, respectively, vs. 296 ± 33 μmol · kg^{-1} · min^{-1} in the wild-type mice; $P < 0.001$). Fat transplantation with or without rosiglitazone treatment increased steady-state glucose infusion rate to the level similar to the wild-type mice (231 ± 17 or 199 ± 31 μmol · kg^{-1} · min^{-1}, respectively).

Basal hepatic glucose production (HGP) showed a tendency to increase in the fatless mice ($P = 0.08$) and was significantly elevated after rosiglitazone treatment in the fatless mice (Fig. 1*A*). Insulin suppressed basal HGP by 80% in the wild-type mice with or without rosiglitazone treatment (Fig. 1*B*). In contrast, insulin suppressed basal

TABLE 1
Metabolic parameters during basal (overnight fasted) and hyperinsulinemic-euglycemic clamp periods in the wild-type, fatless, and fat-transplanted fatless mice with or without 3 weeks of rosiglitazone treatment (Rosi-treated)

			Basal period		Clamp period	
	n	Body weight (g)	Plasma glucose (mmol/l)	Plasma insulin (pmol/l)	Plasma glucose (mmol/l)	Plasma insulin (pmol/l)
Wild-type	6	24 ± 1	7.2 ± 0.7	83 ± 18	6.5 ± 0.6	615 ± 41
Wild-type Rosi-treated	4	23 ± 1	7.1 ± 0.3	89 ± 14	7.4 ± 0.9	690 ± 40
Fatless	5	23 ± 1	17.1 ± 2.1*	72 ± 18	8.6 ± 1.7	715 ± 57
Fatless Rosi-treated	6	23 ± 1	13.7 ± 1.7*†	118 ± 29	9.0 ± 1.1	643 ± 34
Fat-transplanted fatless	7	23 ± 1	9.8 ± 1.0†	111 ± 25	6.8 ± 0.4	684 ± 39
Fat-transplanted fatless Rosi-treated	11	22 ± 1	10.4 ± 1.1†	124 ± 34	6.8 ± 0.5	627 ± 20

*$P < 0.05$ vs. wild-type mice by Duncan's multiple range test; †$P < 0.05$ vs. fatless mice by Duncan's multiple range test.

HGP by 30% in the fatless mice and failed to suppress basal HGP after rosiglitazone treatment in the fatless mice. Fat transplantation restored insulin's ability to suppress basal HGP with or without rosiglitazone treatment (Fig. 1*B*). Insulin-stimulated whole-body glucose turnover was decreased by 40% in the fatless mice but was normalized to the level similar to that of wild-type mice after rosiglitazone treatment ($P = 0.05$ for fatless mice vs. rosiglitazone-treated fatless mice) and/or fat transplantation in the fatless mice (Fig. 1*C*). The rate of insulin-stimulated glucose uptake in skeletal muscle in vivo was estimated using 2-deoxy-D-[1-^{14}C]glucose injection during hyperinsulinemic-euglycemic clamps in awake mice. Insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius) was decreased by 53% in the fatless mice but was normalized after rosiglitazone treatment and/or fat transplantation in the fatless mice (Fig. 1*D*).

Insulin-stimulated whole-body glycolysis was not significantly different among the groups, although it showed a tendency to decrease in the fatless mice and a tendency to increase after rosiglitazone treatment in the fatless mice (Fig. 2*A*). Insulin-stimulated whole-body glycogen/lipid synthesis were decreased by 66% in the fatless mice with or without rosiglitazone treatment and showed a tendency to increase after fat transplantation with or without rosiglitazone treatment (Fig. 2*B*). Similar to the pattern of changes in whole-body glucose metabolism, insulin-stimulated skeletal muscle glycolysis was decreased by 52% in the fatless mice and returned to normal after rosiglitazone treatment and/or fat transplantation (Fig. 2*C*). Insulin-stimulated muscle glycogen synthesis was decreased by 68% in the fatless mice and was normalized after rosiglitazone treatment and/or fat transplantation (Fig. 2*D*). These findings suggest that decreases in insulin-stimulated muscle glucose uptake was partly responsible for decreases in muscle glucose metabolism and that normalization of insulin-stimulated muscle glucose uptake also restored muscle glucose metabolism after rosiglitazone treatment and/or fat transplantation.

Recent studies in IRS-1 and IRS-2 gene–disrupted mice

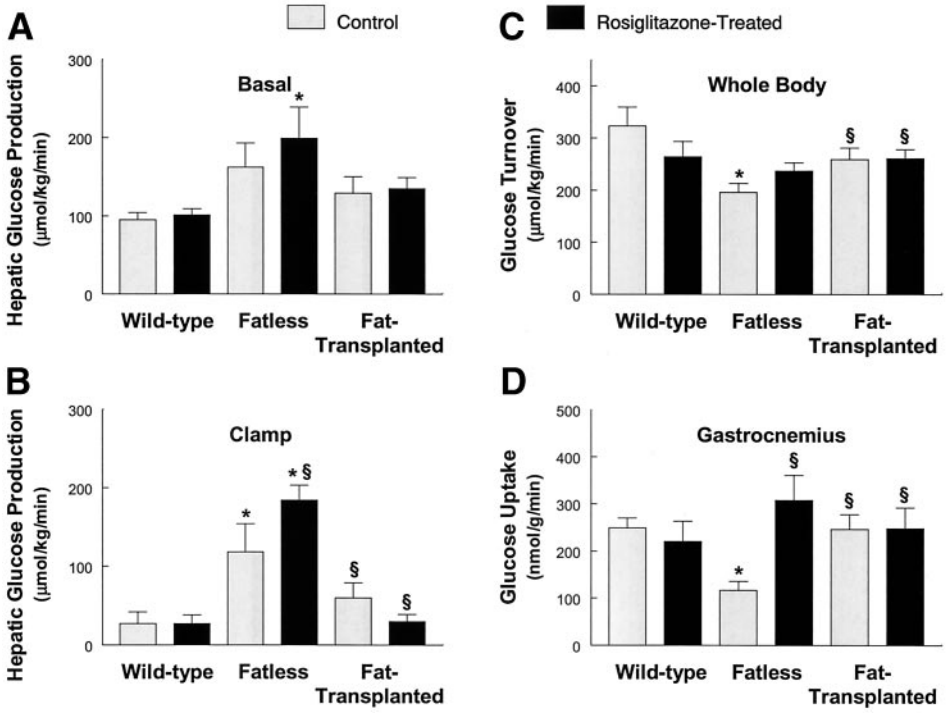

FIG. 1. Liver, whole-body, and skeletal muscle (gastrocnemius) insulin action during hyperinsulinemic-euglycemic clamps in awake wild-type, fatless, and fat-transplanted fatless mice with (black bars) or without (grey bars) rosiglitazone treatment. *A*: Basal hepatic glucose production. *B*: Hepatic glucose production during clamps. *C*: Insulin-stimulated whole-body glucose turnover. *D*: Insulin-stimulated skeletal muscle glucose uptake. Values are means ± SE for 4–12 experiments. *$P < 0.05$ vs. wild-type mice; §$P < 0.05$ vs. fatless mice.

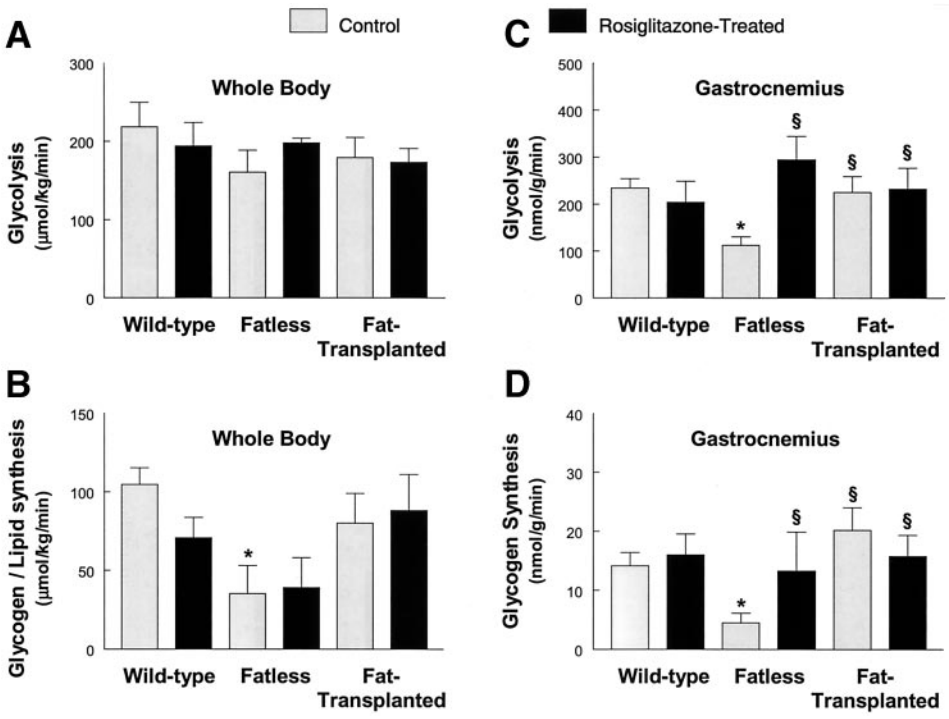

FIG. 2. Insulin-stimulated whole-body and skeletal muscle (gastrocnemius) glucose metabolism in wild-type, fatless, and fat-transplanted fatless mice with (black bars) or without (grey bars) rosiglitazone treatment. *A*: Insulin-stimulated rates of whole-body glycolysis. *B*: Insulin-stimulated rates of whole-body glycogen/lipid synthesis. *C*: Insulin-stimulated rates of skeletal muscle glycolysis. *D*: Insulin-stimulated rates of skeletal muscle glycogen synthesis. Values are means ± SE for 4–12 experiments. *P < 0.05 vs. wild-type mice; §P < 0.05 vs. fatless mice.

have suggested that IRS-1 is important in insulin activation of muscle glucose metabolism (i.e., insulin-stimulated glucose transport and glycogen synthase activity), whereas IRS-2 is more important in mediating insulin activation of hepatic glucose metabolism (i.e., insulin suppression of basal HGP) (25–27). Insulin-stimulated IRS-1 tyrosine phosphorylation in skeletal muscle (gastrocnemius) was decreased by 65% in the fatless mice and returned to normal after rosiglitazone treatment and/or fat transplantation (Fig. 3*A*). Similarly, insulin-stimulated IRS-1–associated PI 3-kinase activity in skeletal muscle (gastrocnemius) was decreased by 66% in the fatless mice and was normalized after rosiglitazone treatment and/or fat transplantation (Fig. 3*B*). Insulin-stimulated IRS-2 tyrosine phosphorylation in liver was decreased by 45% in the fatless mice and showed a tendency to be further reduced after rosiglitazone treatment (Fig. 3*C*). Similarly, insulin-stimulated IRS-2–associated PI 3-kinase activity in liver was significantly decreased by 66% in the fatless mice and showed a tendency to be further reduced after rosiglitazone treatment (P = 0.09; Fig. 3*D*).

To examine whether the changes in insulin signaling and action were associated with changes in tissue-specific fat contents, triglyceride, fatty acyl-CoA, and ceramide con-

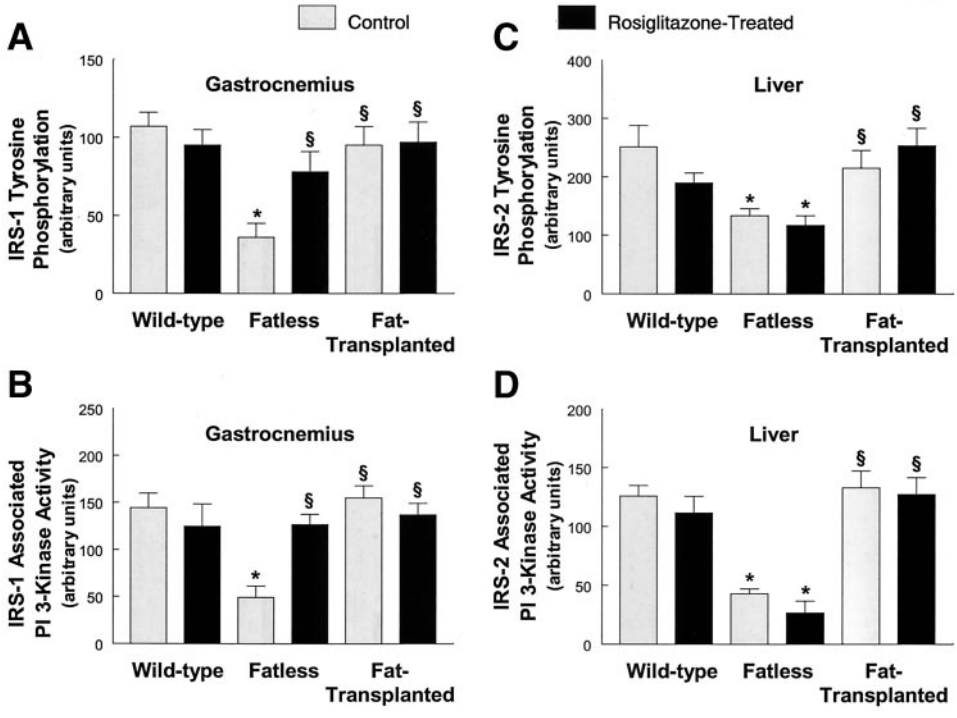

FIG. 3. Insulin signaling in skeletal muscle (gastrocnemius) and liver of wild-type, fatless, and fat-transplanted fatless mice with (black bars) or without (grey bars) rosiglitazone treatment. *A*: Insulin-stimulated IRS-1 tyrosine phosphorylation in skeletal muscle. *B*: Insulin-stimulated IRS-1–associated PI 3-kinase activity in skeletal muscle. *C*: Insulin-stimulated IRS-2 tyrosine phosphorylation in liver. *D*: Insulin-stimulated IRS-2–associated PI 3-kinase activity in liver. Values are means ± SE for 4–12 experiments. *P < 0.05 vs. wild-type mice; §P < 0.05 vs. fatless mice.

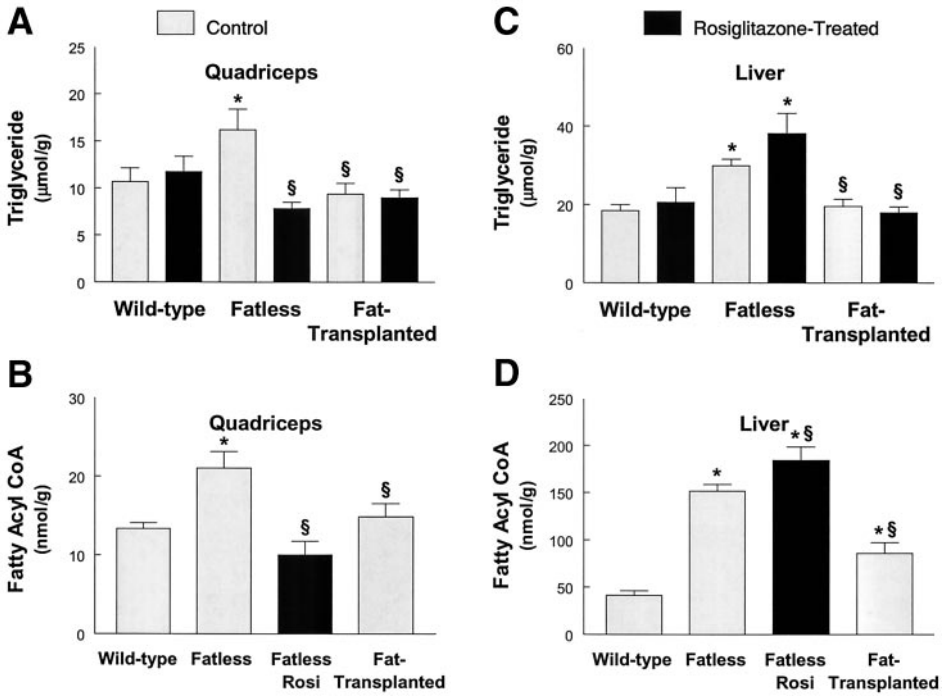

FIG. 4. Intracellular triglyceride and fatty acyl-CoA concentrations in skeletal muscle (quadriceps) and liver of wild-type, fatless, and fat-transplanted fatless mice with (black bars) or without (grey bars) rosiglitazone treatment. *A*: Intramuscular triglyceride concentration. *B*: Intramuscular fatty acyl-CoA concentration. *C*: Intrahepatic triglyceride concentration. *D*: Intrahepatic fatty acyl-CoA concentration. Values are means ± SE for 4–12 experiments. *$P < 0.05$ vs. wild-type mice; §$P < 0.05$ vs. fatless mice.

centrations were measured in skeletal muscle and liver using LC/MS/MS. Triglyceride concentration in skeletal muscle (quadriceps) was increased in the fatless mice and returned to normal after rosiglitazone and/or fat transplantation (Fig. 4*A*). Long-chain fatty acyl-CoA concentration in skeletal muscle (quadriceps) was increased in the fatless mice and returned to normal after rosiglitazone treatment or fat transplantation (Fig. 4*B*). Rosiglitazone treatment or fat transplantation did not affect ceramide concentration in skeletal muscle (quadriceps) of the fatless mice (18 ± 2, 24 ± 4, 17 ± 3, and 19 ± 2 nmol/g in the wild-type, fatless, rosiglitazone-treated fatless, and fat-transplanted fatless mice, respectively). Furthermore, triglyceride concentration in liver was increased in the fatless mice and showed a tendency to increase further after rosiglitazone treatment in the fatless mice ($P = 0.09$; Fig. 4*C*). In contrast, fat transplantation with or without rosiglitazone treatment lowered the liver triglyceride concentration to the level similar to the wild-type mice. Long-chain fatty acyl-CoA concentration in liver was increased in the fatless mice and was further increased after rosiglitazone treatment in the fatless mice (Fig. 4*D*). In contrast, fat transplantation lowered the liver fatty acyl-CoA concentration in the fatless mice. Rosiglitazone treatment or fat transplantation did not affect ceramide concentration in the liver of fatless mice (375 ± 15, 457 ± 44, 377 ± 43, and 383 ± 15 nmol/g in the wild-type, fatless, rosiglitazone-treated fatless, and fat-transplanted fatless mice, respectively).

Insulin-stimulated glucose uptake in tibialis anterior muscle was reduced in the fatless mice but increased after rosiglitazone treatment and/or fat transplantation, consistent with the findings in the gastrocnemius muscles (Fig. 5*A*). Insulin-stimulated glucose uptake in intrascapular brown adipose tissue was increased with rosiglitazone treatment in the wild-type mice, whereas it was decreased in the fatless and fat-transplanted fatless mice with or without rosiglitazone treatment (Fig. 5*B*). Last, insulin-stimulated glucose uptake in epididymal white adipose tissue was unaltered in the wild-type and fat-transplanted fatless mice with or without rosiglitazone treatment (Fig. 5*C*).

DISCUSSION

In this study, we used the A-ZIP/F-1 "fatless" mouse as an adipocyte null model to examine the role of adipocytes in the insulin-sensitizing action of rosiglitazone. Insulin-stimulated glucose uptake in skeletal muscle was significantly decreased in the fatless mice, which was associated with decreased insulin-stimulated tyrosine phosphorylation of IRS-1 and decreased activation of IRS-1–associated PI 3-kinase activity. Because tyrosine phosphorylation of IRS-1 and activation of IRS-1–associated PI 3-kinase are important intracellular insulin signaling events (26–28), these defects may be responsible for subsequent decreases in skeletal muscle glucose uptake in the fatless mice. The defects in muscle insulin signaling and action were accompanied by increased concentrations of triglyceride and fatty acyl-CoA in skeletal muscle of the fatless mice and are consistent with previous studies in animals (29–33) and humans (33–40), demonstrating an inverse relationship between intramyocellular lipid content and insulin sensitivity. It has been hypothesized that the mechanism by which increased levels of fatty acyl-CoA impair skeletal muscle insulin signaling and action involves activation of serine kinase cascade, of which protein kinase C and/or IκB kinase-β may play a role (41–44), leading to the serine phosphorylation of IRS-1 at Ser307 (45). Recent studies have shown that serine phosphorylation of IRS-1 prevents tyrosine phosphorylation of IRS-1 and interferes with its ability to recruit and activate PI 3-kinase, as occurs upon treatment with tumor necrosis factor-α (TNF-α) and okadaic acid (46–49). Transplantation of fat from wild-type littermates into the fatless mice normalized insulin-stimulated glucose uptake and IRS-1–associated PI

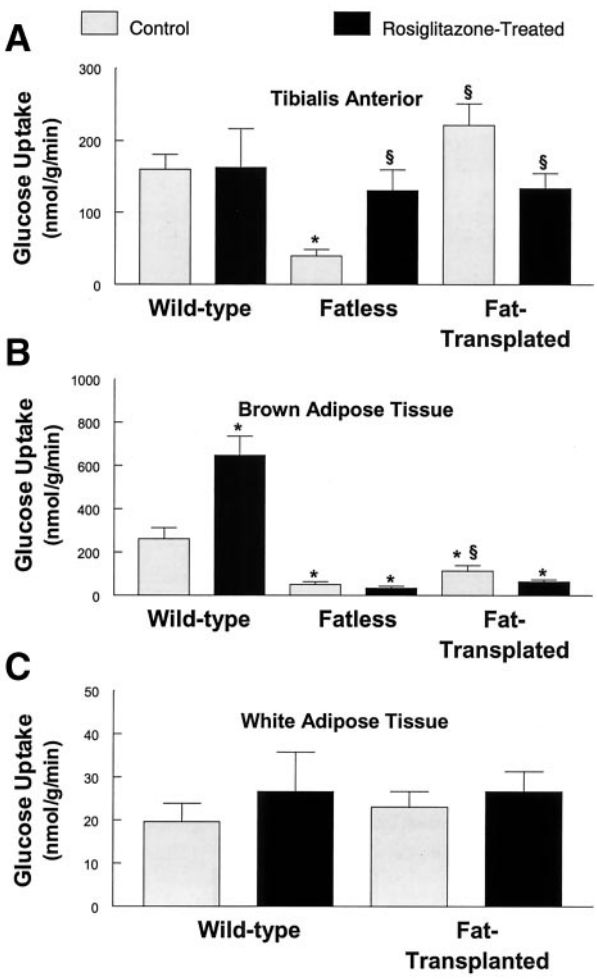

FIG. 5. Insulin-stimulated glucose uptake in tibialis anterior and adipose tissue during hyperinsulinemic-euglycemic clamps in wild-type, fatless, and fat-transplanted fatless mice with (black bars) or without (grey bars) rosiglitazone treatment. *A*: Tibialis anterior. *B*: Intrascapular brown adipose tissue. *C*: Epididymal white adipose tissue. Values are means ± SE for 4–12 experiments. **P* < 0.05 vs. wild-type mice; §*P* < 0.05 vs. fatless mice.

3-kinase activity in skeletal muscle. These changes were accompanied by decreases in skeletal muscle fatty acyl-CoA levels in the fat-transplanted fatless mice. These findings suggest that fat transplantation caused repartitioning of fat away from skeletal muscle and into transplanted adipocytes, and the resultant lowering of fatty acyl-CoA may be responsible for normalization of skeletal muscle insulin action in the fat-transplanted fatless mice.

Rosiglitazone treatment similarly normalized insulin-stimulated glucose uptake and metabolism in skeletal muscle of the fatless mice. This was accompanied by increases in insulin-stimulated tyrosine phosphorylation of IRS-1 and IRS-1–associated PI 3-kinase activity in skeletal muscle of the rosiglitazone-treated fatless mice. Rosiglitazone treatment also decreased triglyceride and fatty acyl-CoA levels in the skeletal muscle of fatless mice. These findings are remarkably similar to what was observed after fat transplantation in the fatless mice and suggest that repartitioning of fat out of skeletal muscle may also be responsible for rosiglitazone's effect on skeletal muscle insulin action. To address this issue further, we examined the effect of rosiglitazone treatment in the fat-transplanted fatless mice and found that rosiglitazone treatment did not further enhance the effect of fat transplantation to lower fatty acyl-CoA content and increase skeletal muscle insulin action in the fatless mice. The lack of additive effect of rosiglitazone and fat transplantation supports the notion that a common mechanism (i.e., repartitioning of fat and fatty acid–derived metabolites) may underlie the effects of rosiglitazone and fat transplantation on normalization of insulin action in the fatless mice. In support of this hypothesis, Mayerson et al. (11) recently showed that 3 months of rosiglitazone treatment in patients with type 2 diabetes resulted in lowering of plasma fatty acids and intrahepatic triglyceride content, which was associated with enhanced insulin action in peripheral adipocytes and an increase in extramyocellular lipid content. Moreover, Sreenan et al. (50) showed that 6 weeks of troglitazone treatment lowered muscle triglyceride content in lean and obese Zucker rats. Taken together, these findings support the hypothesis that rosiglitazone normalized skeletal muscle insulin action in the fatless mice by repartitioning fat and fatty acid–derived metabolites away from the skeletal muscle.

Rosiglitazone was recently shown to upregulate aP2, fatty acid transporter, and lipoprotein lipase, which are key enzymes involved in fatty acid utilization in white adipose tissue, during adipocyte differentiation (8). Activation of these adipocyte enzymes by rosiglitazone may contribute to its ability to promote redistribution of fat out of skeletal muscle and into adipocytes and indirectly affecting muscle insulin sensitivity. The resultant lowering of intramyocellular fat may improve skeletal muscle insulin action. However, because fatless mice are devoid of adipocytes, one obvious question is, "Where does the fat repartition to?" In this regard, insulin's ability to suppress basal HGP was significantly impaired in the fatless mice, and this was accompanied by marked decreases in insulin-stimulated tyrosine phosphorylation of IRS-2 and IRS-2–associated PI 3-kinase activity, which is an important intracellular mediator of hepatic insulin signaling (26,27). In contrast to rosiglitazone's insulin-sensitizing effect on skeletal muscle insulin action, rosiglitazone treatment grossly exacerbated hepatic insulin resistance in the fatless mice. These defects in hepatic insulin signaling and action were associated with increased levels of triglyceride and fatty acyl-CoA in liver of the fatless mice and their sustained levels in the rosiglitazone-treated fatless mice. These data are consistent with the findings of Chao et al. (20) that rosiglitazone treatment caused hepatic steatosis in the A-ZIP/F-1 fatless mice that was due to increased expression of hepatic PPAR-γ. These findings indicate that as a result of an ablation of adipose tissue, rosiglitazone caused a repartitioning of fat away from skeletal muscle and into liver of the fatless mice, thereby improving skeletal muscle insulin sensitivity while exacerbating hepatic insulin resistance in the fatless mice. Thus, rosiglitazone treatment was shown to have a divergent regulation of insulin action in skeletal muscle and liver of the fatless mice. In this regard, this is the first study to demonstrate that rosiglitazone may partition fat into liver in the presence of inadequate fat storage in the adipocytes potentially by stimulating hepatic PPAR-γ. Moreover, because changes in insulin sensitivity were observed in the adipo-

cyte-null A-ZIP/F-1 mice, this study further demonstrates that local accumulation of fat (i.e., fatty acid–derived metabolites) may alter insulin sensitivity in skeletal muscle and liver independent of adipocyte-derived circulating factors (e.g., TNF-α, leptin, resistin, Acrp30) (46,51–53).

It is also possible that rosiglitazone directly alters skeletal muscle insulin sensitivity independent of its PPAR-γ–mediated changes in adipocyte gene expression (54–56). Hevener et al. (54) recently showed that troglitazone prevented fatty acid–induced insulin resistance in skeletal muscle independent of changes in plasma fatty acid levels, although the concentration of intracellular fatty acid–derived metabolites, which are the likely mediators of fatty acid–induced insulin resistance, was not measured. In addition, Brunmair et al. (55) found that TZDs suppressed lipid oxidation in isolated soleus muscle. Further evidence supporting the direct insulin-sensitizing action of TZDs in skeletal muscle comes from a recent observation that TZDs increase skeletal muscle expression of lipoprotein lipase, fatty acid–binding protein, and muscle carnitine palmitoyl transferase-1, which are genes involved in lipid metabolism (18,56). Furthermore, TNF-α is an adipocyte-derived circulating factor that has been demonstrated to regulate negatively skeletal muscle insulin sensitivity, and TZDs were found to prevent TNF-α–induced insulin resistance in skeletal muscle (17).

In summary, rosiglitazone treatment increased insulin action in skeletal muscle of the A-ZIP/F-1 fatless mice by repartitioning fat away from skeletal muscle and into liver and thereby lowering fatty acid–derived metabolites in skeletal muscle, resulting in enhanced insulin signaling. These data support the hypothesis that rosiglitazone treatment enhances insulin action in skeletal muscle mostly through an indirect mechanism by repartitioning fat (and fatty acid–derived metabolites) out of skeletal muscle and into other PPAR-γ responsive tissues.

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