Insulin-Sensitizing Effect of Rosiglitazone (BRL-49653) by Regulation of Glucose Transporters in Muscle and Fat of Zucker Rats

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Thiazolidinediones (TZDs), a class of antidiabetic agents, are specific agonists of peroxisome proliferator activator receptor (PPARγ). However, their mechanisms of action, and the in vivo target tissues that mediate insulin sensitization are not well understood. The aim of this study was to investigate the role of glucose transporters (GLUT-1 and GLUT-4) in the TZD insulin-sensitizer action. The effects of rosiglitazone treatment were studied using Zucker (fa/fa) rats after 7 days of oral dosing (3.6 mg/kg/d). Rosiglitazone lowered (≈ 80%) basal plasma insulin levels in obese rats and substantially corrected (≈ 50%) insulin resistance based upon results from hyperinsulinemic euglycemic clamp studies. GLUT-4 protein levels were reduced (≈ 75%) in adipose tissue of obese rats and treatment with rosiglitazone normalized them. Interestingly, GLUT-1 protein content was increased in adipose tissue (≈ 150%) and skeletal muscle (≈ 50%) of obese rats and treatment with rosiglitazone increased it even more by 5.5-fold in fat and by 2.5-fold in muscle. Consistent with these results, basal (GLUT-1–mediated) transport rate of 3-O-methyl-D-glucose into isolated epitrochlearis muscle was elevated in response to rosiglitazone. Incubation of fully differentiated 3T3-L1 adipocytes with the drug for 7 days increased the levels of GLUT-1 protein, but did not affect GLUT-4 levels. In conclusion, rosiglitazone may improve insulin resistance in vivo by normalizing GLUT-4 protein content in adipose tissue and increasing GLUT-1 in skeletal muscle and fat. While the drug has a direct effect on GLUT-1 protein expression in vitro without a direct effect on GLUT-4 suggests that direct and indirect effects of rosiglitazone on glucose transporters may have an important role in improving insulin resistance in vivo.

THIAZOLIDINEDIONES (TZDS), a new class of oral antidiabetic agents, are high-affinity ligands for peroxisome proliferator-activated receptors (PPARs) and function as insulin sensitizers.[1] Many studies have shown that administration of TZDs to obese animal models of insulin resistance and type 2 diabetes patients can result in a substantial correction of hyperglycemia and/or hyperinsulinemia.[2-6] PPARs constitute a distinct subfamily of the superfamily of nuclear receptors, which are activated by naturally-occurring fatty acids or fatty acid derivatives.[7] Activation of PPARγ by TZDs regulates the transcription of genes involved in preadipocyte differentiation and fat deposition.[8] The PPARγ receptor has 2 protein isoforms PPARγ1 and γ2 derived from alternative splicing.[9] Interestingly, PPARγ2 is found exclusively in adipocytes, whereas PPARγ1 is expressed predominantly in adipocytes, but is also expressed in a variety of tissues including liver, colon, lung, and skeletal muscle, the major site of impaired insulin action in type 2 diabetes and obesity.[10-13] PPARγ expression has been found to be increased in adipose and muscle tissue of some insulin-resistant obese rodents and humans.[14] However, a missing piece of the puzzle is the exact tissue site at which TZDs function to promote insulin action in muscle, the predominant tissue responsible for insulin-mediated glucose disposal. It has been shown that TZDs increase glucose transport activity and GLUT-4 expression in adipose tissue of different animal models,[4,15,16] but it is not clear whether they have a direct effect in skeletal muscle. These changes occur together with a reduction in fasting glucose and insulin levels, so it is uncertain if the effects on transport/transporters are direct or due to the change of metabolic environment. Although there is some evidence suggesting that TZDs can directly increase expression of GLUT-1 in L6 myocytes,[17] human skeletal muscle cells,[18] isolated cardiomyocytes,[19] and skeletal muscle BC3H1 cells,[20] the hypothesis that activation of PPARγ in vivo can directly affect glucose transporters and insulin action in skeletal muscle has not been tested.

In the present report, we examined the in vivo effects the thiazolidinedione, rosiglitazone (BRL-49653), have on glucose metabolism and GLUT-1 and GLUT-4 protein levels in adipose and skeletal muscle tissue in Zucker fatty and Zucker lean rats. We show that rosiglitazone substantially improves peripheral insulin sensitivity in vivo, and this effect is associated with normalization of GLUT-4 protein in fat and an increase of GLUT-1 protein levels not only in fat, but in skeletal muscle with a correspondent effect on glucose transport. Furthermore, we found a direct effect of rosiglitazone to increase GLUT-1 expression in vitro in adipocytes.

MATERIALS AND METHODS

Materials

Dimethyl sulfoxide (DMSO), dexamethasone, 3-isobutyl-1-methylxanthine (IBMIX), and bovine serum albumin (BSA) were from Sigma (St Louis, MO). Microrenethane catheters were purchased from Braintree Scientific (Braintree, MA). Ketamine and xylazine were purchased from J.A. Webster (Sterling, MA). Anti-GLUT-1 polyclonal antibody was a generous gift from Dr Sam Cushman (National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], National Institutes of Health [NIH], Bethesda, MD). Anti-GLUT-4 polyclonal antibody was obtained from Chemicon International (Temecula, CA).

Animals

All surgical and experimental procedures performed for this study were approved by the Institutional Animal Care and Use Committee (IACUC) at Abbott Laboratories. Male obese and lean Zucker rats, approximately 12 weeks of age, were obtained from Harlan Laborato-

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ries (Madison, WI). The rats were single-housed on a 12-hour fixed light-dark cycle and were allowed ad libitum access to water and Purina rodent chow (Purina 5002, St Louis, MO).

Oral Dosing and Hyperinsulinemic/Euglycemic Clamp Technique

Rats were randomized into 4 groups: obese and lean Zucker rats treated with rosiglitazone (n = 8) and obese and lean rats treated with vehicle (0.2% hydroxypropylmethylcellulose (HPMC) (n = 7). The rats were dosed (3.67 mg/kg) daily via oral gavage for 7 days. On day 6, the rats were fasted overnight, and hyperinsulinemic/euglycemic clamps were performed the next day.

Two days before dosing was begun, rats were anesthetized with a cocktail of ketamine (75 mg/kg) and xylazine (2 mg/kg). Two catheters were placed in a jugular vein, 1 for insulin infusion and 1 for glucose infusion. Another catheter was placed in a carotid artery for blood sampling. The catheters were stored in a subcutaneous pocket between the scapule of the rat until the morning of the clamp.

To begin the clamp procedures, the catheters were carefully externalized and connected to the infusion pumps (Harvard Infusion Pumps, Model 22, South Natick, MA). Before the start of the infusions and also at the end, 0.5 mL arterial blood samples were drawn for measurement of plasma insulin. Insulin levels were measured using a radioimmunoassay kit (Linco Research, St Charles, MO). Blood glucose was also measured before the start of the infusion using a Precision G glucose monitor (Medisense, Abbott Laboratories, Abbott Park, IL). Insulin/somatostatin was formulated as follows: 0.1% BSA, 600 mU/mL insulin (Eli Lilly, Indianapolis, IN), 6.0 μg/mL somatostatin (Sigma). This formulation was administered at a constant insulin infusion rate of 10 mU/kg/min. The insulin infusion was started first, followed by a 50% D-glucose solution (Abbott Laboratories) infusion. Blood glucose was measured at 5-minute intervals, and the glucose infusion rate (GIR) was adjusted accordingly to maintain a blood glucose of $\approx$ 100 mg/dL.

Tissue Preparation

Rats were dosed for 7 days with vehicle (n = 28) or rosiglitazone (n = 32) (3.67 mg/kg/d) via oral gavage. On day 8, glucose and plasma insulin concentrations were determined to verify the effect of rosiglitazone in these animals and their *vastus lateralis* muscles, and epididymal fat pads were removed and lysed. Plasma insulin levels decreased in the obese Zucker rats treated with rosiglitazone (7.42 $\pm$ 1.71 ng/mL) in comparison with the vehicle-treated animals (24.20 $\pm$ 3.12 ng/mL), and the response was similar to the changes observed in the rats, which were used for the clamp procedure. Lysis buffer for adipose tissue contained 25 mmol/L Tris-HCl (pH 7.4), 0.5 mmol/L EGTA, 25 mmol/L NaCl, 10 mmol/L sodium fluoride (NaF), 1% NP40, 0.02 mmol/L leupeptin, 1 mmol/L benzamidine, 2 mmol/L 4-(2-aminoethyl) benzene sulfonyl fluoride hydrochloride (AESBF; Calbiochem, La Jolla, CA), and 1 mmol/L Na_3VO_4. The skeletal muscle lysis buffer contained 50 mmol/L HEPES, 137 mmol/L NaCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10 mmol/L $Na_4P_2O_7$, 10 mmol/L NaF, 2 mmol/L EDTA, 1 mmol/L dithiothreitol (DTT), 10% glycerol, 2 mmol/L Na_3VO_4, 1 mmol/L AESBF, 0.1 mg/mL aprotinin, and 1% NP40. Samples were homogenized using a Polytron on a medium speed for approximately 10 seconds. The samples were then centrifuged at 4°C for 30 minutes at 10,000 rpm, and stored at -70°C until immunoblotting techniques were performed.

Cell Culture

3T3-L1 cells (American Type Culture Collection, Rockville, MD) were grown in high-glucose Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic (GIBCO, Grand Island, NY). Confluent 3T3-L1 preadipocytes were induced to differentiate into adipocytes as previously described.[21] Briefly, 1 day after confluence, cells were treated with DMEM containing 10% FBS, 400 nmol/L insulin (Boehringer Mannheim, Indianapolis, IN), 250 nmol/L dexamethasone, and 0.5 mmol/L isobutylmethylxanthine (Sigma) for 3 days. Subsequently, this medium was replaced by DMEM containing 10% FBS. Cells were used for the experiment 14 days after the induction of differentiation. At that time, differentiated 3T3-L1 adipocytes were treated with rosiglitazone at a final concentration of 1 μmol/L for 7 days. Cells serving as controls were treated with 0.1% DMSO. Growth media was changed every 2 days. On day 8, the cells were washed once with phosphate-buffered saline (PBS), lysed, and stored at -70°C until immunoblotting techniques were performed.

Detection of Glucose Transporters by Immunoblotting

Protein concentrations of individual samples were determined using the bicinchoninic acid method (BCA) protein assay method from Pierce (Rockford, IL). Rat samples and 3T3-L1 cell lysates, (40 μg/well), were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 10% Tris-HCL ready gels, transferred to nitrocellulose membranes, immunoblotted using anti-GLUT-1 or anti-GLUT-4 polyclonal antibodies, and detected with enhanced chemiluminescence (Amersham, Piscataway, NJ). The membranes were scanned and quantitated using the FluorImager and Storm 860 with laser densitometer (Molecular Dynamics, Sunnyvale, CA). Quantification of the bands were expressed in arbitrary units.

Glucose Transport

Glucose transport rates were evaluated in isolated epitrochlearis skeletal muscles from obese and lean Zucker male rats ($\approx$ 8 weeks of age) as described.[22] Epitrochlearis muscle was used in these studies instead of the *vastus lateralis* muscle, because it is a flat, thin, with a large surface area to mass ratio, which allows for rapid diffusion of glucose and insulin throughout the muscle. As rats increase in age and body weight, the mass and thickness of the muscle also increases. The much larger muscles obtained from 12-week-old rats present a diffusion barrier to glucose and insulin. Therefore, we intentionally used obese Zucker rats and age-matched lean rats for these experiments, at the earliest possible age. Rats were treated for 7 days prior to transport study with rosiglitazone (3.67 mg/kg, by mouth, twice daily) or 0.2% HPMC, and fasted overnight prior to the experiment. Muscles were obtained and preincubated at 37°C in oxygenated Krebs-Ringer buffer (KRB) pH 7.4, containing 8 mmol/L glucose. Muscles were randomly assigned to groups (n = 6/group) and incubated for an additional 20 minutes in KRB-glucose containing insulin (100 nmol/L) or no addition (basal glucose uptake). Tissues were incubated for a 10-minute washout period at 30°C in KRB containing 8 mmol/L D-mannitol prior to evaluation of transport. Transport of 1 mmol/L ^{3}H-3-O-methyl-D-glucose (3MG) into skeletal muscle cells was evaluated at 30°C for 10 minutes. ^{14}C-D-mannitol (7 mmol/L) was included to label extracellular tissue space. Exposure to insulin was maintained through washout and transport incubations.

Statistical Analysis

Data are expressed as mean $\pm$ SEM. Differences within and between groups were tested by 2-way analysis of variance (ANOVA) factorial tests with $P \leq .05$ (Statview, SAS Institute, Cary, NC).

RESULTS

Effect of Rosiglitazone on Body Weights, Plasma Glucose, and Insulin Concentrations

As indicated in Table 1, the Zucker obese rats that received either vehicle or rosiglitazone had an increase in their body

Table 1. Characteristics of Lean and Obese Zucker Rats Treated With Vehicle or Rosiglitazone

	Obese Vehicle	Obese Rosiglitazone	Lean Vehicle
Body weights (g)			
Pretreatment	424.9 ± 11.6	423.1 ± 7.9	337.5 ± 4.6
Posttreatment	451.4 ± 11.8	457.3 ± 8.5	343.5 ± 5.3
Blood glucose (mg/dL)			
Pretreatment	99.5 ± 11.5	85.8 ± 4.9	95.8 ± 1.5
Posttreatment	95.0 ± 3.0	88.3 ± 6.8	89.0 ± 4.6

NOTE. Data are the mean ± SEM; n = 8 for all groups.

weights compared with Zucker leans. Vehicle-treated and rosiglitazone-treated obese rats increased their body weight by 5.9% and 7.5%, respectively, after 7 days. As indicated in Table 1, there were no significant changes in whole blood glucose in any of the groups after 7 days of treatment with rosiglitazone or vehicle. The Zucker obese rat is hyperinsulinemic, which accounts for the very high pretreatment insulin levels (Fig 1); however, after treatment with rosiglitazone, the insulin levels decreased by 6-fold. Insulin levels did not change significantly in the lean and obese rats after treatment with vehicle.

Clamp Studies

To determine the insulin-sensitizing effect of rosiglitazone in vivo, clamp studies were performed. Figure 2A shows the levels of glucose at each sampling time during the clamp experiment. Approximately 30 minutes after the start of the infusions, the clamp was achieved and subsequently maintained for 1 hour. Figure 2B displays a measure of the glucose infusion rates. The Zucker lean vehicle controls had an average GIR during the clamp of approximately 38 versus the obese vehicle controls, which have an average GIR of 15. The GIR in the obese rats treated with rosiglitazone increased significantly by 2-fold, demonstrating an improvement in peripheral insulin sensitivity.

Effect of Rosiglitazone on Glucose Transporters

To determine if the improvement of peripheral insulin sensitivity observed in the clamp studies was associated with enhanced glucose transporter expression, we measured the protein levels of GLUT-1 and GLUT-4 in adipose tissue and skeletal muscle after treatment with rosiglitazone.

GLUT-4 protein content was significantly reduced (≈ 75%) in adipose tissue of obese rats in comparison with lean rats (Fig 3). Treatment with rosiglitazone for 7 days induced a normalization of GLUT-4 protein content in the obese animals. In contrast, rosiglitazone did not change GLUT-4 levels in lean animals. In skeletal muscle, there was no effect on GLUT-4 protein levels in any of the animal groups (Fig 3).

GLUT-1 protein content was increased in adipose tissue (≈ 150%) and skeletal muscle (≈ 50%) of Zucker obese rats with respect to their lean littermates (Fig 4). Rosiglitazone treatment caused an increase on GLUT-1 protein levels by 5.5-fold in fat and by 2.5-fold in muscle from obese rats, but did not affect GLUT-1 levels in lean animals.

Effect of Rosiglitazone on Skeletal Muscle Glucose Transport

To determine whether the increase on GLUT-1 protein levels could possibly play a role in improving insulin sensitivity after rosiglitazone treatment in skeletal muscle, we measured 3-O-methyl-D-glucose transport in epitroclearis muscles from Zucker obese and lean rats, treated with either vehicle or rosiglitazone (Fig 5). There was a decrease of basal and insulin-stimulated glucose transport in muscle from obese animals in comparison to their lean littermates. Rosiglitazone treatment resulted in an increase of basal glucose transport and a corresponding increase on the insulin-stimulated glucose transport,

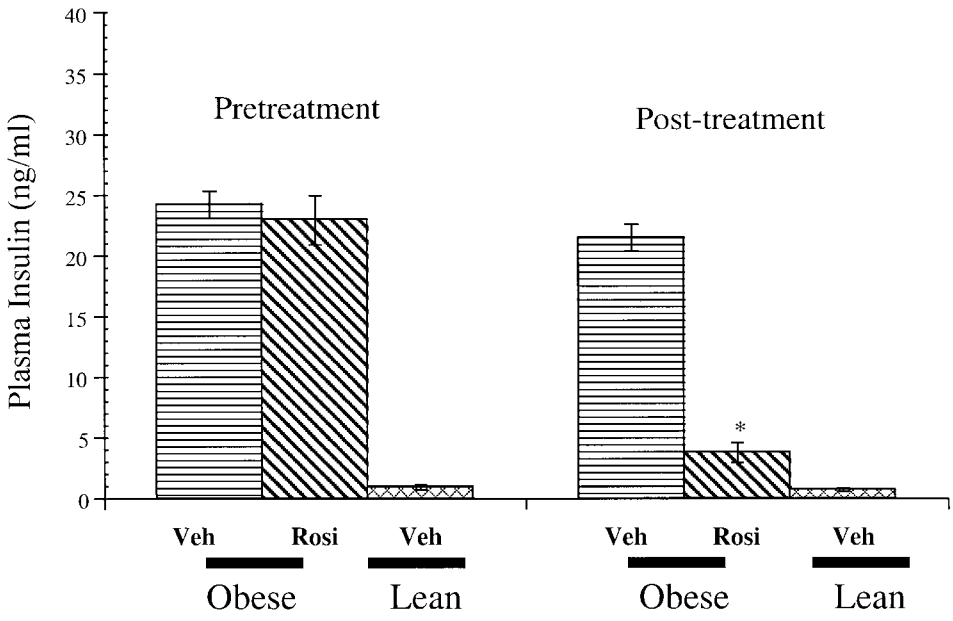

Fig 1. Effect of rosiglitazone on the plasma insulin levels in obese Zucker rats. Rosiglitazone or vehicle were given to obese Zucker rats for 7 days as described. Each point shows the mean ± SEM from 7 rats. *$P <$ .05 from rosiglitazone-treated *v* vehicle-treated obese rats.

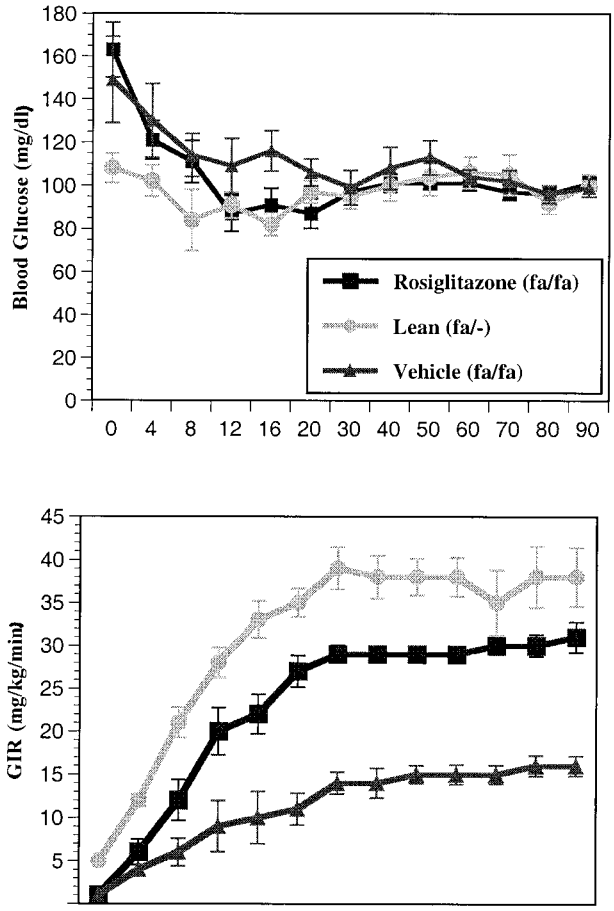

Fig 2. (A) Plasma glucose and (B) exogenous GIR in insulin clamp studies in lean rats (n = 7), vehicle-treated obese rats (n = 7), and rosiglitazone-treated obese rats (n = 8). Data are the mean ± SEM.

while it had no significant effect on glucose transport in muscle from lean animals.

Effect of Rosiglitazone In Vitro

To examine whether rosiglitazone had a direct effect on GLUT-4 and GLUT-1 protein levels, 3T3-L1 adipocytes were treated with rosiglitazone for 7 days, and GLUT-4 and GLUT-1 proteins were examined by immunoblotting. As shown in Fig 6, rosiglitazone had no significant effect on GLUT-4, but increased significantly GLUT-1 protein levels.

DISCUSSION

This study shows that rosiglitazone substantially improves peripheral insulin sensitivity in vivo associated with normalizing GLUT-4 protein in fat and increasing GLUT-1 protein levels in fat and skeletal muscle. Interestingly, this effect on insulin sensitivity was without a significant increase on body weights, although there is a possibility that this 7-day treatment was not long enough to alter weight gain. Moreover, in this case, rosiglitazone improved insulin sensitivity prior to a significant increase in body mass, suggesting that an increase in fat mass was not necessary for this effect. TZDs are known to promote weight gain and increase fat deposition in rodent models,[23,24] although increased adipogenesis per se would not necessarily cause obesity. TZDs may increase fat cell number while simultaneously decreasing fat cell size.[25] In addition, an increased fat cell number per se without an increase in total energy stored would not necessarily lead to insulin resistance.[26]

To determine whether the improvement on peripheral insulin sensitivity was associated with an effect of rosiglitazone on glucose transporters, we measured the protein levels of GLUT-1 and GLUT-4 in fat and muscle. In most insulin-resistant states, such as obesity and type 2 diabetes, GLUT-4 gene expression is reduced in adipose tissue,[27,28] but normal in skeletal muscle.[29] This substantial decrease in GLUT-4 protein levels in adipose tissue of obese rats plays a role in the level of insulin resistance that these rats exhibit. Similarly, in this study, we found a decrease in GLUT-4 protein in fat from obese rats; however, treatment with rosiglitazone normalized it. These findings are compatible with recent reports showing that different TZDs improved glucose tolerance in insulin-resistant rats and mice, and their treatment resulted in an increase of GLUT-4 protein in fat.[4,15,16]

Interestingly, obese rats exhibited an increase in GLUT-1 protein in adipose tissue, and treatment with rosiglitazone increased these levels even more. Recent reports showed that adipose cells from Zucker fatty rats contain 3 times more GLUT-1 protein per cell than Zucker lean rats.[27] This could possibly be a result of GLUT-1 compensating for the low levels

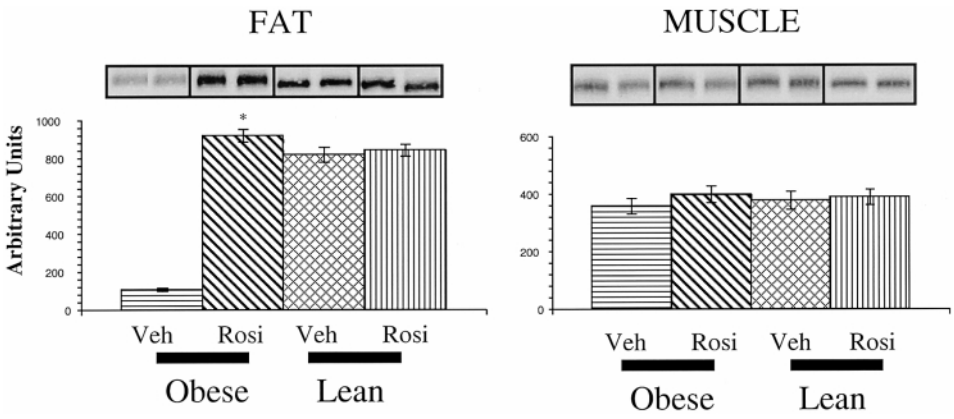

Fig 3. Effect of rosiglitazone on GLUT-4 protein levels in fat and muscle of lean and obese Zucker rats. Representative immunoblot of GLUT-4. Mean ± SEM densitometric analysis of 8 different samples/treatment. *$P < .05$ for rosiglitazone-treated *v* vehicle-treated obese rats.

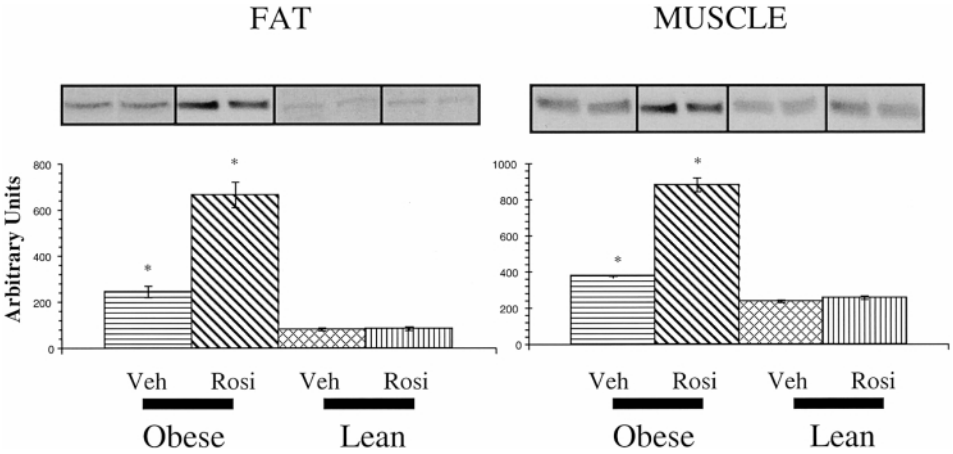

Fig 4. Effect of rosiglitazone on GLUT-1 protein levels in fat and muscle of lean and obese Zucker rats. Representative immunoblot of GLUT-1. Mean ± SEM densitometric analysis of 8 different samples/treatment. *$P < .05$ for rosiglitazone-treated *v* vehicle-treated obese rats.

of GLUT-4 that were found in adipose cells. It has also been reported that in adipose tissue,[30] chronic insulin treatment increases GLUT-1 protein content while causing a decrease of GLUT-4 protein levels; therefore, another explanation for the high level of GLUT-1 and decreased level of GLUT-4 in fat from obese rats could be a secondary effect of the hyperinsulinemia found in these animals. However, rosiglitazone, while reducing insulin levels, increased the protein expression of GLUT-1 in fat and muscle, suggesting the possibility of a direct effect of the drug. In addition, our studies showed that rosiglitazone induced an increase of GLUT-1 protein levels in fully differentiated 3T3-L1 cells, but did not affect GLUT-4. This is in agreement with our preliminary results showing that rosiglitazone increased mainly basal glucose transport in fully differentiated 3T3-L1 adipocytes with a corresponding effect on the insulin-stimulated glucose transport (data not shown). In other studies, it has been shown that troglitazone treatment of fully differentiated adipocytes resulted in upregulation of GLUT-1,

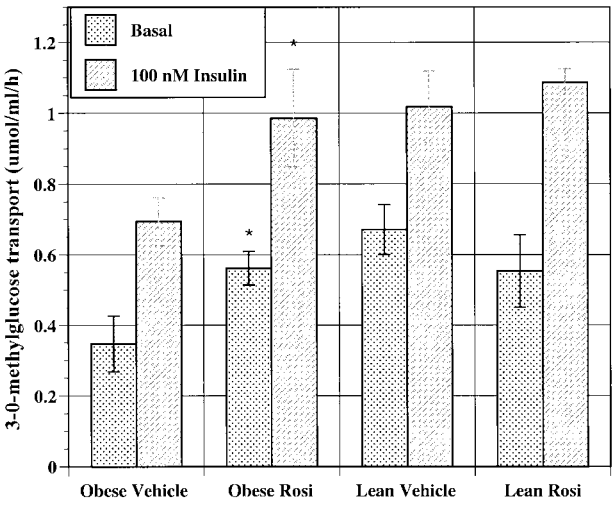

Fig 5. 3-0-Methylglucose transport in epitroclearis muscle from vehicle or rosiglitazone-treated lean and obese Zucker rats. Results are the mean ± SEM (n = 6). *$P < .05$ for rosiglitazone-treated *v* vehicle-treated obese rats.

while GLUT-4 expression was unaltered,[31] further suggesting that changes in GLUT-4 may be indirect responses in adipose cells. These results are in contrast with other reports showing that TZDs increased GLUT-4 mRNA levels and glucose uptake in cultured adipocytes, although this effect was very modest.[7,32] However, these treatments were performed during fibroblastic differentiation into adipocytes, and because GLUT-4 is one of the markers of adipocyte differentiation, it is uncertain whether this result is due to augmented differentiation. In our case, there is a possibility that 7 days might not be a long enough incubation period for rosiglitazone to have an effect in vitro on GLUT-4 or that the effect of PPARγ normalization of GLUT-4 in fat was an indirect effect. In contrast, GLUT-1 levels increased in vivo and in vitro by rosiglitazone, and therefore, this suggests that this drug was exhibiting a direct insulin-sensitizing effect.

PPARγ seems to exhibit its primary effects in adipose tissue, and as we have reported here, rosiglitazone treatment does cause an increase in GLUT-1 and GLUT-4 protein levels in adipose tissue. However, it is not clear how PPARγ agonists (TZDs), ie, rosiglitazone, improve insulin sensitivity in muscle. Normally, adipose tissue contributes to less than 10% of glucose disposal, whereas muscle is responsible for most glucose uptake in the body. Muscle tissue expresses significant amounts of PPARγ under basal conditions,[12,13] and its expression is elevated in fat and muscle from insulin-resistant subjects,[14,33] suggesting an involvement in insulin action and resistance. So there is a possibility that in our studies, rosiglitazone might have a direct effect in skeletal muscle of the obese animals increasing GLUT-1 expression and consequently increasing basal glucose uptake. Alternatively, small amounts of PPARγ in muscle may be sufficient or may be induced during TZD treatment, which could result in a direct PPARγ-mediated response of the muscle to TZDs and insulin sensitization,[34] and this can be an important factor through which insulin controls glucose and lipid metabolism. In addition, a recent report suggest that pioglitazone effects on muscle glucose metabolism could not be due to local adipocyte differentiation,[35] discarding the possibility that conversion of myoblasts into adipocytes under TZDs stimulation is responsible for this effect. Studies in cultures of human skeletal muscle cells showed also that tro-

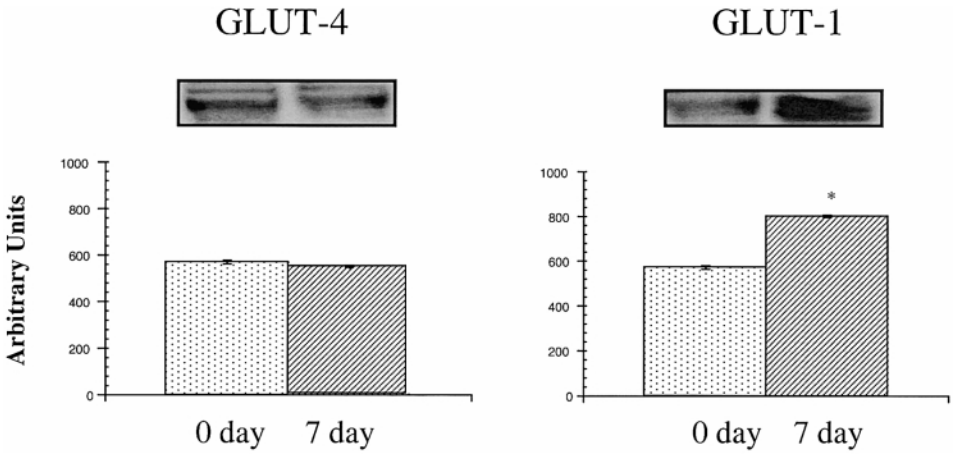

Fig 6. GLUT-4 and GLUT-1 protein levels after treatment of 3T3-L1 adipocytes with rosiglitazone. Results are the mean ± SEM (n = 6). *$P < .05$ for rosiglitazone-treated *v* vehicle-treated cells.

glitazone increased glucose transport activity, as well as GLUT-1 mRNA and protein.[18]

Other important mediators, which can affect insulin sensitivity in muscle, are fatty acids. Increased fatty acid concentrations decrease glucose metabolism in muscle.[36] TZD treatment may increase fatty acid and lipid clearance by adipose tissue without a corresponding increase in fatty acid delivery to muscle.[37] This trapping of fatty acids in fat tissue could possibly lead to a decreased systemic availability and a diminished fatty acid uptake by muscle, potentially improving insulin sensitivity.[36]

Based on these results, we conclude that the levels of GLUT-1 and GLUT-4 are modified in adipose tissue and in muscle cells after treatment with an oral insulin-sensitizing agent, which correlate with the increased peripheral sensitivity observed in vivo. By improving peripheral glucose utilization and by increasing the level of glucose transporters, TZDs could be expected to be very useful in the treatment of type 2 diabetes mellitus in humans. We also propose the possibility that activation of PPARγ in fat and skeletal muscle, the main site of glucose disposal, would provide a direct mechanism to explain TZD action in vivo.

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