Regulation of Glucose Transport and Insulin Signaling by Troglitazone or Metformin in Adipose Tissue of Type 2 Diabetic Subjects

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Type 2 diabetic subjects failing glyburide therapy were randomized to receive additional therapy with either metformin (2,550 mg/day) or troglitazone (600 mg/day) for 3–4 months. Biopsies of subcutaneous abdominal adipose tissue were obtained before and after therapy. Glycemic control was similar with both treatments. Metformin treatment increased insulin-stimulated whole-body glucose disposal rates by 20% ($P < 0.05$); the response to troglitazone was greater (44% increase, $P < 0.01$ vs. baseline, $P < 0.05$ vs. metformin). Troglitazone-treated subjects displayed a tendency toward weight gain (5 ± 2 kg, $P < 0.05$), increased adipocyte size, and increased serum leptin levels. Metformin-treated subjects were weight-stable, with unchanged leptin levels and reduced adipocyte size (to 84 ± 4% of control, $P < 0.005$). Glucose transport in isolated adipocytes from metformin-treated subjects was unaltered from pretreatment. Glucose transport in both the absence (321 ± 134% of pre-Rx, $P < 0.05$) and presence of insulin (418 ± 161%, $P < 0.05$) was elevated after troglitazone treatment. Metformin treatment had no effect on adipocyte content of GLUT1 or GLUT4 proteins. After troglitazone treatment, GLUT4 protein expression was increased twofold (202 ± 42%, $P < 0.05$). Insulin-stimulated serine phosphorylation of Akt was augmented after troglitazone (170 ± 34% of pre-Rx response, $P < 0.05$) treatment and unchanged by metformin. We conclude that the ability of troglitazone to upregulate adipocyte glucose transport, GLUT4 expression, and insulin signaling can contribute to its greater effect on whole-body glucose disposal. *Diabetes* 51: 30–36, 2002

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BSA, bovine serum albumin; DEXA, dual-energy X-ray absorptionometry; HWS, HEPES washing salts; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; PPARγ, peroxisome proliferator–activated receptor γ.

Major characteristics of type 2 diabetes include impaired utilization of glucose and resistance to the ability of insulin to stimulate glucose uptake and disposal in tissue (1). Insulin stimulation of glucose uptake is initiated by the hormone binding to cell surface receptors with activation of the intrinsic tyrosine kinase activity of the receptor (rev. in [2]). Critical events in the subsequent phosphorylation cascades include receptor-mediated tyrosine phosphorylation of insulin receptor substrates (IRSs), especially IRS-1, followed by association of IRS-1 with the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K) and activation of the kinase. The lipid products of PI3K contribute to activation of the serine kinase Akt (protein kinase B), followed by translocation of the insulin-regulated glucose transporter isoform, GLUT4, from intracellular pools to the plasma membrane (3,4). Numerous investigations have established PI3K as essential, if not sufficient, for glucose transport stimulation (3,4), whereas the role of Akt is still controversial (5,6). Each of these events and proteins represent a potential site or cause of insulin resistance.

Glucose intolerance in type 2 diabetes is manifested by defects in glucose transport into muscle (6,7) and adipose tissue (8). In adipose tissue from diabetic subjects, defective glucose transport has been shown to result from a reduced complement of insulin-stimulated GLUT4 in intracellular pools (9). Insulin receptor tyrosine kinase activity is also impaired in adipocytes of obese type 2 diabetic subjects (10). Both the expression and insulin-stimulated phosphorylation of IRS-1 are reduced in adipose tissue from diabetic subjects (11). Events downstream of IRS-1 are also impacted, as insulin stimulation of PI3K activity and phosphorylation of Akt are impaired (11). Thus, adipose tissue insulin resistance in type 2 diabetes involves defects in both insulin signaling pathways and final effector systems, i.e., glucose transporters.

A number of therapeutic approaches have proven useful in controlling hyperglycemia and improving insulin action in type 2 diabetic patients. These include weight loss (12), exercise (13), sulfonylureas (14), biguanides such as metformin (15), and thiazolidinediones (16,17). More recently, combination therapies have been used with increasing frequency and effectiveness (18). Although the general mechanisms by which these various therapies exert their

hypoglycemic effects are known, tissue-specific effects in human type 2 diabetes are less well understood.

In the current study, type 2 diabetic individuals failing control with sulfonylurea therapy had either metformin or the thiazolidinedione troglitazone added to their treatment regimen. The goal was to match the extent of glycemic control and compare the impact of the therapies on previously reported defects in insulin signaling and glucose transport in isolated adipocytes as well as on whole-body glucose disposal and insulin action.

RESEARCH DESIGN AND METHODS

Subjects and treatment protocol. A total of 21 male and female type 2 diabetic subjects (between the ages of 30 and 70 years) who were poorly controlled (HbA_{1c} >8.5% and fasting plasma glucose >140 mg/dl) on maximal doses of any sulfonylurea agents were recruited. Except for diabetes, the subjects were healthy and on no other medications known to influence glucose metabolism. After being screened, their existing sulfonylurea medication was discontinued, and all subjects were uniformly started on glyburide 10 mg b.i.d. for 4 weeks. Baseline studies were performed, and then the subjects were randomized to either the troglitazone or metformin treatment group. Treatment involved troglitazone titration of up to 600 mg per day or metformin up to 2,550 mg per day for 4–6 weeks as required to achieve glycemic goals. After 3–4 months of troglitazone or metformin treatment, patients were readmitted for repeat studies. Subjects were counseled to consume a fixed calorie diet for the duration of the study. Insulin action was determined by a 3-h hyperinsulinemic (300 mU · m^{-2} · min^{-1})–euglycemic (5.0–5.5 mmol/l) clamp, and the glucose disposal rate was measured during the last 30 min of the clamp (19). Glucose and insulin (20) levels were determined with standard techniques. Serum leptin levels were measured by radioimmunoassay (21). The experimental protocol was approved by the Committee on Human Investigation of the University of California, San Diego. Informed written consent was obtained from all subjects after the protocol was explained.

Dual-energy X-ray absorptionometry scanning. Whole-body composition was determined by dual-energy X-ray absorptionometry (DEXA) scanning (22). Anthropometric measurements of skinfold thickness and waist-to-hip ratio were performed by a single experienced individual.

Materials. Human biosynthetic insulin was kindly supplied by Eli Lilly (Indianapolis, IN). Collagenase was purchased from Worthington (Freehold, NJ). Bovine serum albumin (BSA; Cohn fraction V) was obtained from Roche (Indianapolis, IN). [^{14}C]3-*O*-methylglucose was purchased from New England Nuclear (Boston, MA). Antibodies were obtained from the following sources: GLUT1 and GLUT4 were from Biogenesis (Kingston, NH), pS473-Akt was from New England Biolabs (Beverly, MA), IRS-1 and p85 were from Upstate Biotechnology (Sarnac Lake, NY), Akt 1/2 and p110β were from Santa Cruz Biotechnology (Santa Cruz, CA), and peroxisome proliferator–activated receptor-γ (PPAR-γ) was from Biomol (Plymouth Station, MA). Horseradish peroxidase–conjugated anti-mouse and anti-rabbit IgGs were from Amersham (Arlington Heights, IL), and SuperSignal-enchanced chemiluminesence substrate was from Pierce (Rockford, IL). Electrophoresis reagents were purchased from Bio-Rad (Richmond, CA). All other chemicals were reagent grade and purchased from Sigma (St. Louis, MO).

Adipose tissue biopsy and preparation of human adipocytes. Adipose tissue was obtained by needle biopsy of the lower subcutaneous abdominal depot using a 5-mm side-cutting needle. Lidocaine (1%) was infiltrated in a square-field fashion, and the biopsy was taken from the center of the field. Isolated adipocytes were prepared by a modification (8) of the method of Rodbell (23). After digestion and filtration, the cells were washed twice in a buffer consisting of 150 mmol/l NaCl, 5 mmol/l KC1, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l $CaC1_2$, 2.5 mmol/l NaH_2PO_4, 10 mmol/l HEPES, and 2 mmol/l pyruvate (pH 7.4), supplemented with 4% BSA. The cells were then divided, and one portion was washed twice more in the same buffer and resuspended at ~2–5 × 10^5 cells/ml for glucose transport assay. The major portion of the cells were washed twice in a buffer (HEPES washing salts [HWS]) consisting of 116 mmol/l NaCl, 5 mmol/l KCl, 0.5 mmol/l $MgSO_4$, 0.7 mmol/l $CaCl_2$, 25 mmol/l HEPES, 5 mmol/l glucose, and 2% BSA (pH 7.4) and resuspended at ~1 × 10^5 cells/ml before cell extraction.

Cell counts were performed by a modification of method III of Hirsch and Gallian (24), in which cells were fixed in 2% osmium tetroxide and counted with a model ZB Coulter Counter (Coulter Electronics, Hialeah, FL) equipped with a 400-μm aperture tube. Osmium fixed adipocytes were sized using a light microscope with a calibrated ocular scale. Diameters of 150–200 cells were measured for calculation of cell volume.

3-*O*-methylglucose transport. Adipocytes (~5 × 10^5 cells/ml) were incubated for 60 min at 37°C in the absence or presence of insulin (8.5 nmol/l). Transport activity was assessed by measuring the initial rates of uptake of 3-*O*-methylglucose (15–20 μmol/l), using a modification (8) of the method of Whitesell and Gliemann (25).

Adipocyte treatment and extraction. Adipocytes in HWS were incubated for 50 min at 37°C with gentle shaking. Cells were treated with or without insulin for 15 min at 37°C and then rapidly washed twice in 17°C, insulin-free, BSA-free HWS buffer as previously described (26). A twice-concentrated solubilization buffer was then added: final concentrations were −20 mmol/l Tris-HCl, 145 mmol/l NaCl, 10% glycerol, 5 mmol/l EDTA, 1% Triton X-100, 0.5% NP-40, 200 μmol/l sodium orthovanadate, 200 μmol/l phenylmethylsulfonyl fluoride, 1 μmol/l leupeptin, 1 μmol/l pepstatin, and 10 mg/ml aprotinin (pH 7.5). After lysis/extraction for 30 min at 4°C with repeated vortexing, nonsolubulized material was removed by centrifugation at 14,000*g* (10 min, 4°C), and the total cell extracts were stored at −70°C before analysis.

Electrophoresis and Western blotting. Procedures for the electrophoresis, transfer, and Western blotting of proteins are similar to standard methods (27). Detection was by enhanced chemiluminescence followed by densotometric analysis. Quantitation of the blots was performed using ScanAnalysis software (Biosoft, Cambridge, U.K.). An internal standard, prepared from adipocytes of a single subject, was included in each gel to permit for correction of variability between blots.

Statistical analysis. Statistical analysis was performed using the StatView II program (Abacus Concepts, Berkeley, CA). Statistical significance was evaluated with Students's t test and repeated measures analysis of variance. Paired analysis was used to determine the effect of treatment in the same subject. Data are presented as means ± SE. Significance was accepted at the $P < 0.05$ level. Because of limitations in tissue availability, not all analyses could be performed in all subjects. The number of subjects studied is given in the table and figure legends.

RESULTS

Experimental subjects and in vivo effects of treatment. After initial screening, type 2 diabetic subjects failing control on glyburide alone were randomized into two groups and fully characterized, including analysis of body composition by waist/height measurements, skinfold thickness, DEXA scanning, hyperinsulinemic-euglycemic clamps, and adipose tissue biopsies. At baseline, the subjects in the two groups were matched for age, obesity (BMI), and other clinical characteristics, including insulin action on whole-body glucose disposal (Table 1). Subjects then had either metformin or troglitazone added to their treatment regimen, and the studies were repeated 3–4 months later. Posttreatment HbA_{1c} levels were comparable in the two groups: 7.0 ± 0.4 vs. 7.5 ± 0.3% for troglitazone and metformin, respectively (P = NS). In addition, relative changes in fasting glucose, HbA_{1c}, and insulin levels were the same in both groups (Table 1), further indication that the extent of glycemic control was matched between groups. Insulin-stimulated whole-body glucose disposal improved with metformin treatment, by 20 ± 9% over the paired baseline value ($P < 0.05$). The improvement in insulin action after troglitazone treatment (44 ± 7%, $P < 0.01$) was significantly greater ($P < 0.05$) than the relative change in the metformin group (Table 1).

The metformin subjects maintained their weight (Table 1). Their body composition, as determined by percent fat from DEXA analysis, was also stable (posttreatment value = 99 ± 5% of baseline, P = NS). Serum leptin levels were unaltered with metformin treatment (Table 1). Even with a preservation of overall adiposity, there was a significant reduction in the average size of subcutaneous adipocytes (16 ± 4% decrease, $P < 0.005$) in the metformin treatment group. Conversely, there was a modest but statistically significant weight gain in the troglitazone-treated group (Table 1), whereas percent body fat (104 ±

TABLE 1
Effects of treatment on clinical characteristics of type 2 diabetic subjects

	Troglitazone (n = 10)		Metformin (n = 11)	
	Pre-Rx	Change from pre-Rx (%)	Pre-Rx	Change from pre-Rx (%)
Age (years)	56 ± 2		55 ± 2	
Weight (kg)	110.4 ± 5.4	4 ± 1*	96.3 ± 8.3	0 ± 1
BMI (kg/m^2)	36.3 ± 2.2	4 ± 1*†	32.2 ± 2.8	−0.3 ± 1.0
Fasting glucose (mmol/l)	10.7 ± 0.5	−36 ± 7*	12.2 ± 0.7	−31 ± 6*
Fasting insulin (pmol/l)	246 ± 42	−18 ± 9*	222 ± 36	−14 ± 6*
Fasting leptin (ng/ml)	15.7 ± 7.7	24 ± 15	11.1 ± 3.6	−2 ± 6
HbA_{1c} (%)	8.6 ± 0.3	−19 ± 4*	9.2 ± 0.4	−17 ± 4*
Glucose disposal rate (mg · kg^{-1} · min^{-1})	5.21 ± 0.71	44 ± 7*†	6.15 ± 0.48	21 ± 9*

Data are means ± SE. *P < 0.05 vs baseline for same treatment group; †P < 0.05 vs change in metformin treatment group.

5% of baseline, P = NS) did not change. Serum leptin levels increased (24 ± 15%), but not significantly (P < 0.1). There was a trend for fat cell volume to also increase with troglitazone treatment (30 ± 25%, P < 0.1), although this difference also did not attain statistical significance. Initially, subcutaneous adipocyte volumes were similar in the two groups (635 ± 56 vs. 596 ± 25 pL for troglitazone and metformin groups, respectively, P = NS). After treatment, fat cells from the troglitazone-treated group were significantly larger (P < 0.05) than those in the metformin group.

Adipocyte glucose transport and transporter expression. In the baseline studies, adipocytes from both groups were well matched for glucose transport activity in both the absence and presence of added insulin (Fig. 1). Acute insulin exposure caused a 2- to 2.5-fold increase in transport activity in both groups. Metformin treatment had no consistent effect on adipocyte glucose transport in either the absence (29 ± 32% increase over baseline) or presence (83 ± 81% increase, P = 0.35) of insulin. Troglitazone treatment resulted in large increases in both basal (221 ± 133% over baseline, P < 0.05) and insulin-stimulated (318 ± 161%, P < 0.05) glucose transport (Fig. 1). The relatively greater increase in insulin-stimulated transport activity translated into an elevation of insulin responsiveness after troglitazone treatment, from an average 2.3-fold stimulation at baseline to 3.2-fold at posttreatment.

The total cellular complements of adipocyte GLUT1 and GLUT4 were determined by Western blotting. Metformin had no effect on either GLUT1 (19 ± 30% over baseline, not shown) or GLUT4 (9 ± 15% below baseline) (Fig. 2) expression in adipocytes. With troglitazone, there was a tendency for GLUT1 protein to increase (64 ± 42%, P = 0.16, not shown) and a doubling in GLUT4 expression

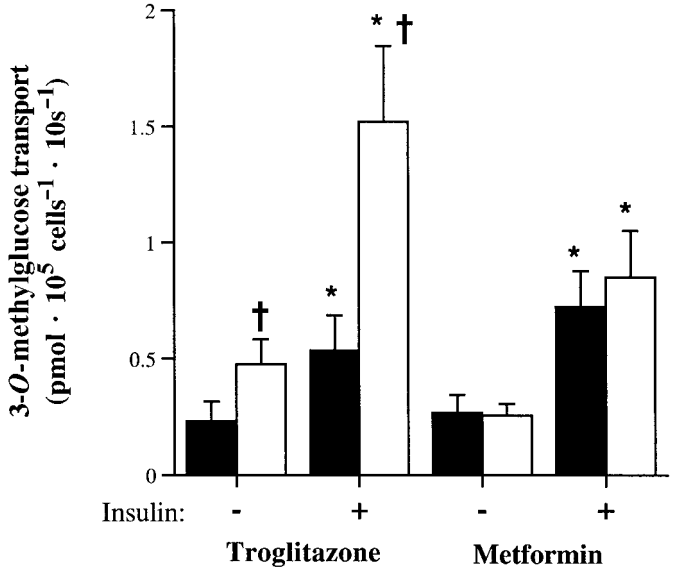

FIG. 1. Effect of treatment on glucose transport into adipocytes. Absolute rates of 3-*O*-methylglucose transport were measured in adipocytes obtained before (■) and after (□) drug treatment with acute (60 min) incubation in the absence (−) or presence (+) of insulin (8.2 nmol/l). Results are average + SE, n = 10 for the troglitazone group and n = 11 for the metformin group. *P < 0.05 vs. absence of insulin for same treatment condition; †P < 0.05 vs. paired pre-Rx value.

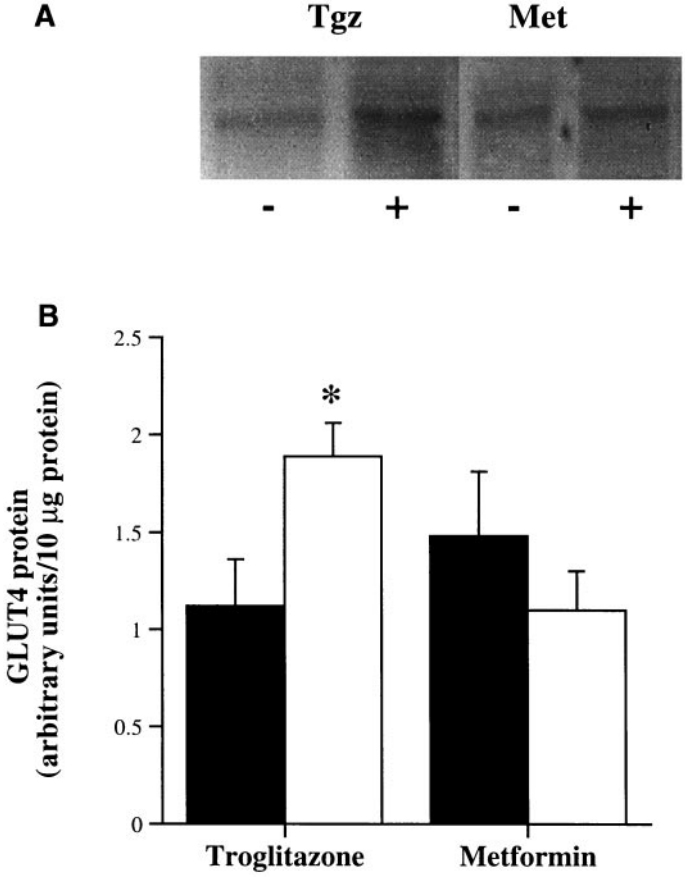

FIG. 2. Effect of treatment on GLUT4 glucose transporter protein expression in adipocytes. Adipocytes were isolated from subcutaneous abdominal adipose tissue obtained before (baseline, ■) and after (□) treatment. *A*: Representative Western blot. *B*: Quantitation of blots, normalized to total extract protein expression. Results are average + SE, n = 10 for the troglitazone group and n = 11 for the metformin group. *P < 0.05 vs. paired pre-Rx value.

TABLE 2
Effects of treatment on signaling protein expression

Protein (arbitrary units/10 μg protein)	Troglitazone (n = 7)		Metformin (n = 11)	
	Pre-Rx	Post-Rx	Pre-Rx	Post-Rx
IRS-1	7.4 ± 1.8	8.5 ± 2.8	10.0 ± 1.4	9.6 ± 1.7
p85α	5.4 ± 0.7	7.5 ± 1.2	7.2 ± 1.8	6.0 ± 0.9
p110β	4.5 ± 1.3	5.1 ± 1.5	3.9 ± 0.5	4.0 ± 0.8
Akt	4.2 ± 1.1	5.9 ± 2.0	5.4 ± 1.8	5.1 ± 1.0

Data are means ± SE.

(102 ± 44% over baseline, $P < 0.05$) (Fig. 2). The changes, or lack thereof, in GLUT1 and GLUT4 expression mirror those seen in basal and insulin-stimulated glucose transport activities (Fig. 1).

Insulin signaling. To determine what impact glycemic control might have on insulin signaling, the expression of key components in a pathway leading to glucose transport stimulation were measured by Western blotting. Baseline expression of the proteins of interest did not differ significantly between groups (Table 2). Neither metformin nor troglitazone treatment had any consistent or significant effect on the total cellular protein expression of IRS-1, p85α, p110β, or Akt 1/2 (Table 2).

Limitations of tissue availability prevented analysis of insulin receptor kinase and PI3K activities. However, Ser^{473} phosphorylation of Akt, a reliable downstream indicator of activation of the enzyme (28) that was also impaired in adipocytes from diabetic subjects (11), was determined. Because S^{473} phosphorylation of Akt is routinely undetectable in the absence of insulin (Fig. 3), the results are presented as the amount of pS473-Akt in the insulin-stimulated condition and normalized to the amount of Akt protein in the same sample. Insulin exposure resulted in the generation of a phosophorylated form of Akt to a comparable extent in both groups at baseline. Insulin stimulation of Akt phosphorylation in adipocytes was unaltered after metformin treatment (Fig. 3). However, the insulin effect was increased (70 ± 34% over baseline response, $P < 0.05$) with troglitazone treatment.

Modulation of PPAR-γ expression. The nuclear receptor PPAR-γ has been identified as a target for troglitazone and other thiazolidindiones (29). PPAR-γ protein expression was determined by Western blotting with an antibody that recognizes a region common to the γ1 and γ2 isoforms. Adipocyte content of PPAR-γ was similar in the troglitazone and metformin groups before treatment (14.2 ± 5.8 vs. 14.5 ± 4.5 arbritrary units/10 μg protein for troglitazone and metformin groups, respectively). Neither treatment had any consistent effect on PPAR-γ protein expression; there was a 32 ± 69% increase over control with troglitazone and a 50 ± 52% increase over control with metformin (P = NS for both).

DISCUSSION

Glucose intolerance in type 2 diabetes is the consequence of a number of defects, including impaired insulin secretion by the pancreatic β-cell, resistance of peripheral tissues to the glucose-utilizing effects of insulin, and augmented hepatic glucose production (rev. in [1]). Pharmacological approaches have been developed to treat each of these problems. Combination therapy with these agents is an increasingly popular approach because multiple defects are often present in diabetic subjects, and many individuals respond only partially to single agents. Any understanding of the molecular mechanism(s) by which multiple therapies act to improve glycemic control is complicated by common therapeutic consequences. Questions include which responses might be specific for a certain drug on a particular tissue and which are the result of generalized changes in glucose and insulin levels. These issues were addressed in type 2 diabetic subjects who failed sulfonylurea therapy when the dose of troglitazone or metformin was titrated to attain a common therapeutic target. We were able to achieve this goal of matched glycemic control because changes in glucose, insulin, and HbA_{1c} levels were similar in the troglitazone and metformin treatment groups (Table 1). Despite the comparable glycemic control, troglitazone treatment was twice as effective as metformin in improving whole-body insulin action and glucose disposal, suggesting that factors other than changes in circulating glucose and insulin levels are responsible for the difference. Other investigators have also found a greater efficacy of troglitazone when compared with metformin on glucose disposal in type 2 diabetic subjects (30).

A common consequence of tight glycemic control is weight gain, although this appears to occur less frequently with metformin (18). Our results are in agreement with this behavior, as troglitazone-treated subjects demon-

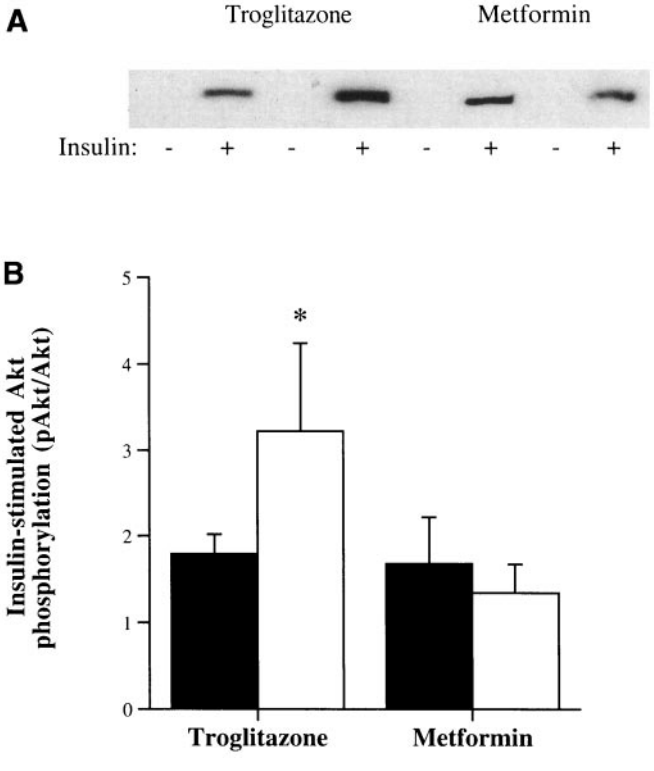

FIG. 3. Influence of in vivo treatment on insulin-stimulated Akt serine phosphorylation in adipocytes. Cell extracts were prepared from adipocytes obtained before (■) and after (□) drug treatment with acute (15 min) incubation in the absence (−) or presence (+) of insulin (8.2 nmol/l). *A*: Representative Western blot. *B*: Quantitation of pS473-Akt in insulin-treated cells normalized to total Akt 1/2 protein in the same sample. Results are average + SE, n = 7 for both the troglitazone and metformin groups. *P < 0.05 vs. paired pre-Rx value.

strated modest weight gain, whereas metformin-treated subjects did not. One postulated mechanism by which troglitazone could increase insulin action even in the presence of weight gain is augmented apoptosis of large, insulin-resistant adipocytes coupled with proliferation and differentiation (31) of smaller, more insulin-sensitive adipocytes. Although evidence in support of this hypothesis has been obtained in rodents (32), our results in humans are the opposite, showing a trend toward an increase in cell size in subcutaneous adipose tissue with troglitazone treatment. A more likely explanation may be a redistribution of fat stores, as several investigators have found thiazolidinedione treatment to reduce visceral fat mass and increase subcutaneous fat (33,34). That supposition would be supported by the lack of any significant effect of troglitazone treatment on whole-body fat content; more of it may now be in the subcutaneous depots, a subtle distribution that could not be detected by changes in waist-to-hip ratio or abdominal skinfold thickness. Although we did not directly measure the mass of the different adipose depots, an increase in adipocyte size from subcutaneous fat would be consistent with an increase in the size of that depot.

Although a tight relationship between adiposity and serum leptin levels is a common observation (35), the effects of antidiabetic therapy on leptin in the absence of weight loss are more mixed. In agreement with the lack of effect seen in the current study, short-term metformin treatment did not influence leptin levels in type 2 diabetic women (36). The effects of treatment on leptin levels in the current report are in general agreement with the behavior of body weight: a tendency toward increases in both parameters with troglitazone and no change after metformin.

Even though the major portion of insulin-mediated glucose disposal occurs in skeletal muscle, a strong relationship between whole-body glucose disposal and glucose transport into adipocytes has been demonstrated (8). A similar tendency, although not statistically significant ($P = 0.18$), was observed in the current subjects. Adipocytes from individuals with type 2 diabetes display defects in glucose transport (8) and GLUT4 expression as well as insulin-stimulated IRS-1–associated PI3K activity and Akt phosphorylation (11)—behaviors that are also found, for the most part, in diabetic skeletal muscle (6,7,37). Thus, in many ways, insulin resistance in diabetic adipocytes is reflective of that in skeletal muscle. Troglitazone treatment was able to reverse many of the major defects identified in diabetic adipocytes as well as in impaired glucose transport, reduce GLUT4 expression, and reduce insulin stimulation of Akt phosphorylation. If S473 phosphorylation of Akt is taken as an indirect downstream marker of PI3K activity, a commonly accepted supposition (28), then the results suggest that troglitazone treatment also improved insulin-stimulated PI3K activity. Thus, troglitazone treatment ameliorates diabetes-related impairments in both insulin signaling and final effector systems. None of these defects were influenced by metformin treatment. There are reports of thiazolidinediones improving adipocyte glucose uptake and GLUT4 expression in various animal models of insulin resistance (38,39), results that mirror the current findings in human adipocytes and suggest that the effects are general to the class of thiazolidinediones and not specific for troglitazone.

The ability of metformin to reduce hyperglycemia through effects on hepatic glucose production has been well documented both in vivo (40,41) and in vitro (16). The mechanisms for this action include both reduced gluconeogenesis and increased glyogenesis (16). Information on metformin actions on glucose disposal in other tissues is more varied. Metformin has been reported to upregulate GLUT1 expression in human skin fibroblasts (39). Conversely, metformin had no effect on GLUT1 or GLUT4 expression in skeletal muscle of obese (*fa/fa*) Zucker rats (42). The question of direct effects of metformin on glucose disposal in nonhepatic tissues versus the consequences of relieving glucotoxicity remains to be resolved.

The ability of troglitazone to increase whole-body glucose uptake has been demonstrated in a number of insulin-resistant animal models (43,44) as well as in subjects with type 2 diabetes (17,45). This response is observed in the absence or presence of sulfonylureas, although, just as we found, thiazolidinedione treatment augments glycemic control over that seen with sulfonylurea monotherapy (46,47). The fact that both troglitazone (46) and rosiglitazone (47) gave a similar result indicates that this is a general effect of thiazolidinediones and not limited to troglitazone. Treatment of insulin-resistant animals with several different thiazolidinediones have been shown to increase glucose transport and/or GLUT4 expression in adipocytes (48). Treatment of fully differentiated 3T3-L1 adipocytes with troglitazone, which is more reflective of the therapeutic situation in adult diabetic subjects, resulted in an increase in basal glucose uptake and upregulation of GLUT1 expression that was similar to certain aspects of our findings in adipocytes. In addition, we have reported that troglitazone treatment of human skeletal muscle cells in culture also caused increases in GLUT1 expression (49), implicating control of glucose transporter expression as a key contributor to the antihyperglycemic actions of this family of drugs.

Thiazolidinediones are thought to exert their glucose and lipid fatty acid–lowering actions by activating PPAR-γ (29), thereby altering the transcription of genes involved in glucose and lipid metabolism. Expression of PPAR-γ is greatest in adipose tissue (29), and it has been proposed that adipose tissue is a major target for thiazolidinedione action. Increases in adipose tissue GLUT4 expression after troglitazone treatment can certainly explain the augmented glucose transport into adipocytes that those subjects displayed. As there were no changes in the expression of several key proteins involved in insulin activation of PI3K and Akt, the mechanism responsible for increased insulin-stimulated phosphorylation of Akt is uncertain but may involve control of an inactivating phosphatase or proteins involved in intracellular localization of signaling complexes. The fact that the changes in adipocytes reported here are specific for troglitazone and do not occur in response to metformin suggest that they involve mechanisms beyond correction of glucotoxicity and hyperinsulinemia, as those changes were common to both treatments. The troglitazone effect is most likely occurring through activation of PPAR-γ (31) and not through upregulation of the expression of the receptor.

Several conclusions can be drawn from the current results. One is that although troglitazone and metformin may be equally effective in controlling hyperglycemia, they do so by different mechanisms and by acting on different tissues. The greater efficacy of troglitazone to increase glucose disposal, as opposed to reducing glucose production, is due, at least in part, to the ability of the thiazolidinedione to increase glucose uptake and insulin action in adipose tissue. The relationship between increased glucose uptake in subcutaneous adipocytes and further improvements in whole-body glucose disposal, occurring in response to troglitazone and not metformin, also suggests that adipose tissue can make a major contribution to whole-body glucose disposal.

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