

# Mechanism of Troglitazone Action in Type 2 Diabetes

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To examine the metabolic pathways by which troglitazone improves insulin responsiveness in patients with type 2 diabetes, the rate of muscle glycogen synthesis was measured by  $^{13}\text{C}$ -nuclear magnetic resonance (NMR) spectroscopy. The rate-controlling steps of insulin-stimulated muscle glucose metabolism were assessed using  $^{31}\text{P}$ -NMR spectroscopic measurement of intramuscular glucose-6-phosphate (G-6-P) combined with a novel  $^{13}\text{C}$ -NMR method to assess intracellular glucose concentrations. Seven healthy nonsmoking subjects with type 2 diabetes were studied before and after completion of 3 months of troglitazone (400 mg/day) therapy. After troglitazone treatment, rates of insulin-stimulated whole-body glucose uptake increased by  $58 \pm 11\%$ , from  $629 \pm 82$  to  $987 \pm 156 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$  ( $P = 0.008$ ), which was associated with an  $\sim 3$ -fold increase in rates of insulin-stimulated glucose oxidation (from  $119 \pm 41$  to  $424 \pm 70 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ;  $P = 0.018$ ) and muscle glycogen synthesis ( $26 \pm 17$  vs.  $83 \pm 35 \mu\text{mol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ ;  $P = 0.025$ ). After treatment, muscle G-6-P concentrations increased by  $0.083 \pm 0.019 \text{ mmol/l}$  ( $P = 0.008$  vs. pretreatment) during the hyperglycemic-hyperinsulinemic clamp, compared with no significant changes in intramuscular G-6-P concentrations in the pretreatment study, reflecting an improvement in glucose transport and/or hexokinase activity. The concentrations of intracellular free glucose did not differ between the pre- and posttreatment studies and remained  $>50$ -fold lower in concentration ( $<0.1 \text{ mmol/l}$ ) than what would be expected if hexokinase activity was rate-controlling. These results indicate that troglitazone improves insulin responsiveness in skeletal muscle of patients with type 2 diabetes by facilitating glucose transport activity, which thereby leads to increased rates of muscle glycogen synthesis and glucose oxidation. *Diabetes* 49:827-831, 2000

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APE, atom percent enrichment; DEXA, dual-energy X-ray absorptiometry; FFA, free fatty acid; G-6-P, glucose-6-phosphate; GCMS, gas chromatography-mass spectrometry; GCRC, General Clinical Research Center; IRS, insulin receptor substrate; NMR, nuclear magnetic resonance; PPAR, peroxisome proliferator-activated receptor.

The thiazolidinediones represent a new class of oral agents for treatment of type 2 diabetes that improves glycemic control by increasing insulin sensitivity in target tissues, such as muscle and adipose tissue (1-8). Although it is clear that troglitazone works mostly by increasing insulin sensitivity in the periphery, the mechanism(s) by which this occurs remain obscure (2,3,8). In cultures of skeletal muscle, troglitazone has been shown to have acute effects on glucose uptake and chronic effects on glycogen synthase activity (6,9). These effects were accompanied by increases in GLUT1 mRNA and protein but no changes in GLUT4 or glycogen synthase mRNA or protein (6). To date, there have been no studies examining troglitazone action directly in human skeletal muscle in vivo. In the current study, we used  $^{13}\text{C}/^{31}\text{P}$ -nuclear magnetic resonance (NMR) spectroscopy to noninvasively assess the rates of muscle glycogen synthesis combined with indirect calorimetry to assess the effects on rates of whole-body glucose oxidation. To determine the effects of troglitazone on the key rate-controlling steps of muscle glycogen metabolism, we also used  $^{31}\text{P}$ -NMR spectroscopy to measure intracellular glucose-6-phosphate (G-6-P) concentration (10) and a novel  $^{13}\text{C}$ -NMR technique to measure intracellular glucose concentration (11).

## RESEARCH DESIGN AND METHODS

**Subjects.** Seven nonsmoking healthy type 2 diabetic subjects (6 men and 1 woman aged  $55 \pm 4$  years, BMI  $32 \pm 1 \text{ kg/m}^2$ ) with a mean fasting plasma glucose concentration of  $10.9 \pm 1.1 \text{ mmol/l}$ , a lactate concentration of  $1.72 \pm 0.02 \text{ mmol/l}$ , a plasma insulin concentration of  $90 \pm 1 \text{ pg/ml}$ , a plasma C-peptide concentration of  $704 \pm 70 \text{ pg/ml}$ , and a  $\text{HbA}_{1c}$  concentration of  $10.1 \pm 0.6\%$  (normal range 5.3-8.2) were studied. At the time of study enrollment, 3 patients were on a diet-controlled regimen (taking no medications), and the rest were taking sulfonylurea agents, which were discontinued 10 days before the baseline study. Baseline data from 3 of the subjects have previously been reported (11). For 3 days before each of the studies, the subjects were given an isocaloric diet (35 kcal/kg; 60% carbohydrate, 20% protein, and 20% fat) prepared by the metabolic kitchen of the Yale-New Haven Hospital General Clinical Research Center (GCRC). On the third day, the patients were admitted to the GCRC at 4:00 P.M., given dinner at 6:00 P.M., and fasted until the baseline study the following day, as described below.

**Troglitazone treatment.** At completion of the baseline clamp study, the patients were given their first daily dose of 400 mg of troglitazone with dinner before discharge. They were seen by the study nurse every 2 weeks when new medication was dispensed, and they underwent a monthly physical examination and blood work, including liver function tests (aspartate aminotransferase/alanine aminotransferase) and measurements of plasma glucose and  $\text{HbA}_{1c}$  levels, during the 3-month study period. One subject was studied after 6 months of treatment because of recovery after a nonrelated illness that occurred during the third month of the study. Of the 10 patients initially enrolled, 3 were excluded from the study because they were considered nonresponders, as determined by the lack of change from baseline levels in fasting and postprandial glucose concentrations. **Body composition.** On the day of admission, dual-energy X-ray absorptiometry (DEXA) scan (Hologic QDR-4500 W; Hologic, Bedford, MA) was performed with

the subject in the supine position. Fat and lean body mass in each arm, each leg, the trunk, and the head were calculated assuming 17% brain fat and lean body mass consisting of 73% water (12).

**Hyperglycemic-hyperinsulinemic clamp.** At 6:00 A.M. on the day after admission to the GCRC, Teflon intravenous catheters were inserted into an antecubital vein in each arm for blood collection and infusions. The subjects were then brought in a wheelchair to the magnetic resonance center and placed in an NMR spectrometer (2.1 Tesla Bruker Biospec Spectrometer; Bruker, Billerica, MA) with the calf of the right leg positioned over a concentric surface coil as previously described (11,13). Baseline concentrations of muscle glycogen and G-6-P were measured with <sup>13</sup>C- and <sup>31</sup>P-NMR spectroscopy, respectively, and 30 min of indirect calorimetry using the ventilated hood technique (Deltatrack Metabolic Monitor; Sormedics, Anaheim, CA) was performed to measure basal fasting rates of glucose and lipid oxidation, as described earlier (14). At time 0, an infusion of somatostatin (3.5 μmol · m<sup>-2</sup> · min<sup>-1</sup>) was initiated and continued throughout the study to suppress endogenous release of insulin. After 5 min, a primed-continuous infusion of insulin (Humulin; Eli Lilly, Indianapolis, IN) (240 pmol · m<sup>-2</sup> · min<sup>-1</sup>) was initiated to raise plasma insulin concentrations to ~420 pmol/l. Simultaneously, a primed-variable infusion of dextrose (1.1 mol/l) containing 20–60% [<sup>1-13</sup>C]glucose (Cambridge Isotopes, Cambridge, MA) was initiated to raise and maintain plasma glucose concentrations at ~11 mmol/l for the 250-min duration of the clamp study. During the first 60 min of the clamp, changes in G-6-P concentrations were measured. Thereafter, the subject was taken out of the spectrometer, and the NMR probe was changed to a probe that was optimized for measurement of [<sup>13</sup>C]glucose. The patient was then repositioned in the spectrometer, and, at 90 min, a baseline measurement of [<sup>13</sup>C]glucose was begun. At 120 min, an infusion of [<sup>1-13</sup>C]mannitol (99% <sup>13</sup>C enriched) (Cambridge Isotopes, Cambridge, MA) was initiated. Muscle glycogen concentrations were measured from 120 to 220 min. During the final 20–30 min of the study, [<sup>13</sup>C]glucose spectra were obtained for the measurement of intracellular glucose concentrations. This protocol was followed in 4 of the 7 subjects. In the 3 other patients, muscle glycogen and G-6-P concentrations were measured with <sup>13</sup>C/<sup>31</sup>P-NMR spectroscopy throughout the clamp study without infusion of [<sup>1-13</sup>C]mannitol or measurements of intracellular [<sup>13</sup>C]glucose concentrations. During the clamp, blood samples were taken for measurement of plasma glucose concentrations every 5 min, for plasma [<sup>13</sup>C]glucose enrichment every 15 min, and for plasma concentrations of insulin, free fatty acids (FFAs), and lactate every 30 min. Insulin-stimulated rates of glucose and lipid oxidation were measured with indirect calorimetry from 90 to 120 min.

After the 3 months of treatment and the 3 days of ingesting a controlled diet that was identical in composition to the pretreatment diet, the subjects were readmitted to the GCRC, and the hyperinsulinemic-hyperglycemic clamp study was repeated.

**Analyses.** Plasma glucose concentration was measured by the glucose oxidase method with a Beckman Glucose Analyzer (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma immunoreactive insulin concentrations were measured with a double-antibody radioimmunoassay (Diagnostic System Labs, Webster, TX). Plasma FFA concentrations were determined using a microfluorimetric method (15). The <sup>13</sup>C atom percent enrichment (APE) of plasma glucose was determined by gas chromatography–mass spectrometry (GCMS) after plasma glucose was derivatized as the penta-acetate, followed by Ba(OH)<sub>2</sub>/ZnSO<sub>4</sub> deprotonation and semipurification by anion/cation exchange chromatography (AG1-X8, AG50W-X8; Bio-Rad Laboratories, Richmond, CA), as described previously (16). GCMS analysis was performed with a Hewlett-Packard (Palo Alto, CA) 5890 gas chromatograph (HP-1 capillary column, 12 m × 0.2 mm × 0.33 μm film thickness) interfaced to a Hewlett-Packard 5971A Mass Selective Detector operating in the positive chemical ionization mode with methane as reagent gas. Gas chromatography conditions were isothermal at 200°C, and ions with m/z 331, 332, and 333 were monitored for glucose C1 through C6 APE.

**Continuous indirect calorimetry.** Rates of basal and insulin-stimulated rates of glucose and lipid oxidation were measured by the ventilated hood technique using a Deltatrack Metabolic Monitor (Sormedics, Anaheim, CA) as described (14).

**NMR spectroscopy.** During the measurements, the subjects were lying inside the NMR spectrometer (Bruker Biospec Spectrometer; 1 m bore 2.1T) with the gastrocnemius muscle of the right leg positioned on top of a concentric surface coil probe for <sup>31</sup>P-, <sup>13</sup>C-, and <sup>1</sup>H-NMR acquisition. Decoupling and shimming were performed as earlier described (10). <sup>31</sup>P-NMR spectra were acquired during the baseline and the first hour of the clamp in 15-min blocks consisting of 288 scans with 4,096 data points. Concentrations of metabolites were calculated from the data as described (10). The area of the β resonance of ATP was used as an internal concentration standard and assumed to represent a concentration of 5.5 mmol/kg of muscle (17). The <sup>31</sup>P-NMR G-6-P measurement has been validated in an animal model by comparison with chemical measurements of G-6-P in muscle frozen in situ (18). <sup>13</sup>C-NMR spectra of the C1 glycosyl unit of intramuscular glycogen were obtained during the baseline period (preclamp) and

from 120 to 220 min of the clamp study. Each 15-min block consisted of 4,800 scans using a 90° pulse at coil center and a repetition time of 120 ms. Intramuscular glycogen concentrations were determined by comparison with an external standard glycogen solution (19). <sup>13</sup>C-NMR spectra were processed by methods that have been described in detail in several of our earlier studies (19). The <sup>13</sup>C-NMR technique for assessing intramuscular glycogen concentrations has been validated in situ in frozen rabbit muscle (20) and by comparison with biopsies from human gastrocnemius muscle tissue (21). Baseline <sup>13</sup>C-NMR spectra of glucose were obtained from 90 to 120 min, after which the infusion of [<sup>1-13</sup>C]mannitol was initiated. Final measurements of [<sup>13</sup>C]glucose and [<sup>13</sup>C]mannitol were obtained during the final 20–30 min of the clamp study as earlier described (11). The relative concentrations of plasma [<sup>1-13</sup>C]glucose and [<sup>1-13</sup>C]mannitol were determined by <sup>13</sup>C-NMR spectroscopy in a 500 MHz Spectrometer (Bruker Instruments, Billerica, MA), and <sup>13</sup>C enrichment of plasma glucose and mannitol were determined by GCMS (11).

#### Calculations

**Hyperglycemic-hyperinsulinemic clamp.** The rates of glucose infusion during the clamp were calculated in 20-min blocks from 60 to 220 min, were corrected for urinary glucose and glucose space, were averaged for the entire clamp study, and were expressed as micromoles of glucose per meter squared per minute.

**Indirect calorimetry.** The nonprotein respiratory quotients for 100% oxidation of fat and for oxidation of carbohydrates were 0.707 and 1.00, respectively (14). Nonoxidative glucose metabolism was calculated by subtracting the amount of glucose oxidized from the total amount of glucose infused.

**Muscle glycogen synthesis.** Increments in muscle glycogen concentration were calculated from the change in [<sup>1-13</sup>C]glycogen concentration and the plasma [<sup>1-13</sup>C]glucose APE, as described earlier (19). The rate of muscle glycogen synthesis was calculated from the slope of the least squares linear fit to the glycogen concentration curve from 60 to 240 min.

**G-6-P concentrations.** Increments in G-6-P (ΔG-6-P) were calculated as the difference in the concentrations of intramuscular G-6-P between the baseline of each clamp study and the final concentration measured at the end of the clamp.

**Intracellular glucose concentrations.** Intracellular glucose concentrations and intra- to extracellular volumes were determined from comparison of <sup>13</sup>C-NMR spectra of muscle and plasma concentrations of glucose and mannitol as previously described (11,22). Because the <sup>13</sup>C-NMR glucose signal represents both intra- and extracellular glucose, the intracellular glucose concentration was calculated by comparison of the plasma [<sup>13</sup>C]glucose and the [<sup>13</sup>C]mannitol signal ratio with the same ratio in the muscle <sup>13</sup>C-NMR glucose signal.

## RESULTS

**Body composition.** There were no changes in body weight or body composition over the 3 months of troglitazone treatment as assessed by DEXA (body weight 95.3 ± 4.5 vs. 91.8 ± 5.1 kg, *P* = 0.34; lean body mass 62.1 ± 3.9 vs. 62.6 ± 2.4 kg, *P* = 0.77; fat mass 30.1 ± 3.8 vs. 26.3 ± 3.7 kg, *P* = 0.15; and percent body fat 31.5 ± 3.4 vs. 27.9 ± 2.7, *P* = 0.12).

**Plasma concentrations of hormones and metabolites.** At the completion of the 3 months of troglitazone treatment, fasting glucose plasma concentrations decreased by 23 ± 4% (*P* = 0.02), and insulin concentrations decreased by 28 ± 1% (*P* = 0.0004). Plasma FFA concentrations decreased by 31 ± 2% (*P* = 0.0027), and there was a decrease in fasting glucagon concentrations of 9 ± 1% (*P* = 0.01) (Table 1). There was a

TABLE 1  
Plasma hormone and metabolite concentrations before and after 3 months of troglitazone treatment

	Before treatment	After treatment	<i>P</i>
Glucose (mmol/l)	10.9 ± 1.2	8.6 ± 0.8	0.020
Insulin (pmol/l)	93 ± 2	67 ± 5	<0.0004
FFA (μmol/l)	748 ± 26	514 ± 1	0.003
Glucagon (ng/l)	63 ± 2	57 ± 2	0.010
C-peptide (nmol/l)	268 ± 66	233 ± 25	0.420
HbA <sub>1c</sub> (%)	10.1 ± 0.6	9.5 ± 0.4	0.190

Data are means ± SE.

TABLE 2

Metabolic parameters during the hyperglycemic-hyperinsulinemic clamp before and after 3 months of troglitazone treatment

	Before treatment	After treatment	<i>P</i>
Glucose infusion rate ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ )	629 $\pm$ 82	987 $\pm$ 156	0.008
Glucose oxidation ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ )	119 $\pm$ 41	424 $\pm$ 70	0.018
Lipid oxidation ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ )	403 $\pm$ 26	210 $\pm$ 44	0.012
Nonoxidative glucose metabolism ( $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ )	486 $\pm$ 93	556 $\pm$ 135	0.436

Data are means  $\pm$  SE.

trend toward a decrease in fasting concentrations of C-peptide and HbA<sub>1c</sub> levels after troglitazone treatment (Table 1).

**Glucose metabolism and glucose infusion rates.** Insulin-stimulated rates of glucose metabolism increased by  $58 \pm 11\%$  from  $629 \pm 81 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$  before the start of troglitazone treatment to  $987 \pm 156 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$  after troglitazone treatment (Table 2). Rates of basal glucose oxidation did not change between the pre- and posttreatment studies, whereas rates of insulin-stimulated glucose oxidation increased from  $119 \pm 41$  to  $424 \pm 70 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$  ( $P = 0.018$ ) (Table 2). Insulin-stimulated rates of lipid oxidation decreased by  $48 \pm 13\%$  ( $P = 0.012$ ) compared with pretreatment. There was a slight but not significant trend for rates of nonoxidative glucose metabolism to increase after troglitazone treatment.

**Rates of muscle glycogen synthesis.** Fasting concentrations of intramuscular glycogen were similar before and after troglitazone treatment ( $53 \pm 4$  vs.  $55 \pm 6 \text{ mmol/l}$ ,  $P = 0.9$ ). Rates of insulin-stimulated muscle glycogen synthesis increased by  $\sim 3.2$ -fold from  $26 \pm 17 \mu\text{mol}/(\text{l muscle} \cdot \text{min})$  during the pretreatment clamp to  $83 \pm 35 \mu\text{mol}/(\text{l muscle} \cdot \text{min})$  during the posttreatment study ( $P = 0.025$ ) (Fig. 1).

**G-6-P concentrations.** Fasting concentrations of intramuscular G-6-P remained unchanged by the troglitazone treatment ( $0.109 \pm 0.027 \text{ mmol/l}$  before treatment vs.  $0.117 \pm 0.016 \text{ mmol/l}$  after treatment,  $P = 0.75$ ). During the pretreatment clamp study, G-6-P concentrations did not increase significantly above baseline ( $\Delta\text{G-6-P } 0.026 \pm 0.009 \text{ mmol/l}$ ). After troglitazone treatment, there was an  $\sim 3$ -fold increase in the increment of intramuscular G-6-P concentrations compared with that of pretreatment ( $\Delta\text{G-6-P } 0.083 \pm 0.019 \text{ mmol/l}$ ,  $P = 0.008$  vs. pretreatment) (Fig. 2).

**Intracellular concentrations of glucose.** Intracellular concentrations of glucose during the pretreatment hyperglycemic-hyperinsulinemic clamp were  $-0.09 \pm 0.16 \text{ mmol/l}$  and were not significantly different from those during the posttreatment hyperglycemic-hyperinsulinemic clamp after 3 months of troglitazone treatment ( $0.05 \pm 0.24 \text{ mmol/l}$ ,  $P = 0.59$  vs. pretreatment).

## DISCUSSION

In this study, we examined the effects of 3 months of troglitazone treatment on rates of insulin-stimulated muscle glycogen synthesis and glucose oxidation in patients with type 2 diabetes. We found that the improvement in insulin-stimulated glucose uptake in the periphery could be attributed to an  $\sim 3$ -fold increase in the rates of insulin-stimulated muscle glycogen synthesis and whole-body glucose oxidation.

To examine the mechanism by which troglitazone caused this improvement in insulin-stimulated muscle glycogen synthesis and glucose oxidation, we combined measurements of intramuscular G-6-P concentrations, as assessed by  $^{31}\text{P}$ -NMR spectroscopy, with measurements of intracellular glucose concentrations, as assessed by use of a novel  $^{13}\text{C}$ -NMR method.

G-6-P is an intermediate between glycogen synthesis and glucose transport/hexokinase, and any decrease in glycogen synthesis activity relative to glucose transport/hexokinase activity would be expected to result in an increase in the G-6-P concentration. Our results show that G-6-P concentrations remained low during the hyperglycemic-hyperinsulinemic clamp, before troglitazone treatment was initiated. This finding is consistent with both the majority of rate control that occurs during glucose transport/hexokinase and our previous results (10,23).

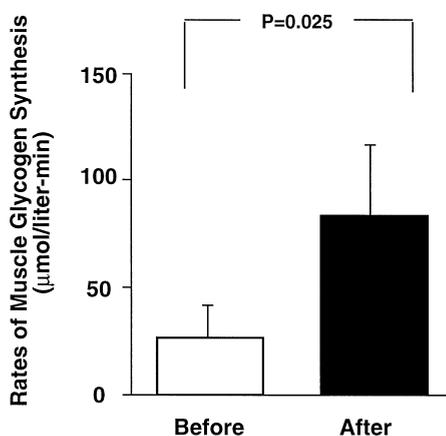


FIG. 1. Rates of insulin-stimulated muscle glycogen synthesis before and after 3 months of troglitazone treatment.

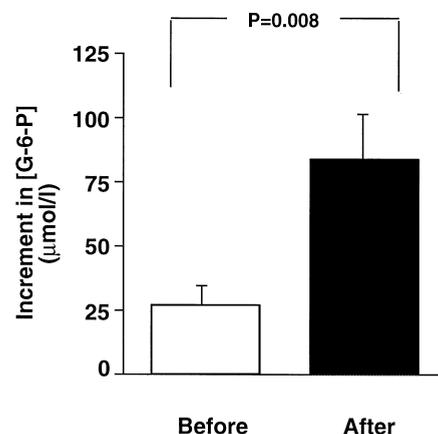


FIG. 2. Increments of intramuscular G-6-P concentrations during the clamp before and after 3 months of troglitazone treatment.

In the same manner, intracellular glucose reflects relative activities of glucose transport and hexokinase. If hexokinase was rate-controlling for muscle glucose uptake, intracellular glucose concentrations would be expected to accumulate to >6 mmol/l (11).

Until recently, it has been difficult to measure the concentration of intracellular glucose by using traditional biopsy techniques due to destruction of the tissue integrity and contamination of the biopsy sample by blood and nonmuscle constituents. Given that a punch biopsy typically yields ~50 mg of tissue, ~10 mg of which represents extracellular space, contamination of the biopsy sample by a single 20- $\mu$ l drop of blood (with plasma glucose concentration of ~5 mmol/l) would almost double the total amount of glucose present in the sample and increase the estimate of intracellular glucose from 0.1 to 1.35 mmol/l. To make these same measurements under hyperglycemic conditions, as performed in this study, would lead to a further doubling of this error. This simple calculation highlights the difficulty in making this measurement with the traditional biopsy technique. The use of NMR spectroscopy, which is noninvasive and therefore maintains intra- and extracellular compartment integrity, combined with mannitol, as a marker of the extracellular space, eliminates both the problem of mixing compartments and the resultant errors associated with the biopsy technique. By use of this approach, we found that the intracellular glucose concentrations remained <0.10 mmol/l during the clamp, which is >50-fold lower than what would be expected if hexokinase were rate-controlling. Furthermore, this finding is consistent with the majority of rate control for insulin-stimulated activation of glycogen synthesis in patients with type 2 diabetes residing at glucose transport (11).

After 3 months of troglitazone treatment, we found an ~3-fold increase in the insulin-stimulated increment in intramuscular G-6-P concentration compared with before therapy associated with no change in intracellular glucose concentrations. These data are consistent with the hypothesis that troglitazone improves insulin action mostly through increasing glucose transport activity. This hypothesis would also be consistent with the observed 3-fold increase in glucose oxidation and muscle glycogen synthesis that was observed in these patients after treatment.

These findings are consistent with studies by Park et al. (6), who examined the effects of troglitazone in isolated muscle strips and found an improvement in glucose uptake independent of changes in glycogen synthase activity. The authors concluded that the insulin mimetic effects of troglitazone were due to enhanced activity and/or translocation of GLUT1 and/or GLUT4. No changes in total cellular content of either transporter isoform were found after acute troglitazone exposure. When muscle strips were incubated with troglitazone for 4 days, both insulin-dependent and insulin-independent muscle glucose uptake improved, which is consistent with an effect on both GLUT1 and GLUT4 by troglitazone.

The ability of thiazolidinediones to augment glucose transport may be dependent in part on protein synthesis through increases in transporter expression (6). Such effects may be mediated through peroxisome proliferator-activated receptors (PPARs), specifically the PPAR- $\gamma$  isoform, a nuclear receptor for which troglitazone has recently been identified as a ligand (24,25). In the muscle cells from type 2 diabetic subjects, the main effect of chronic troglitazone exposure was an increase

in glucose transport and glycogen synthase activity independent of insulin. However, cultured human muscle cells are only modestly insulin responsive; therefore, insulin-stimulated glucose transport would be expected to play a relatively minor role in these cells, compared with the situation in vivo (6).

It is also possible that the improvement in insulin-stimulated muscle glucose metabolism in these diabetic patients was due to troglitazone-induced alterations in FFA metabolism. Troglitazone treatment for 3 months resulted in a 30% decrease in fasting plasma FFA concentrations ( $P = 0.003$ ), which is consistent with findings in several other studies (1–4). Over the past few years, it has become increasingly evident that FFAs play a prominent role in promoting insulin resistance (26–29). In a recent study, we demonstrated that an acute elevation of plasma FFA levels reduced insulin-stimulated muscle glycogen synthesis by ~50% through a reduction in glucose transport activity. We also demonstrated that these changes were associated with reduced levels of insulin receptor substrate (IRS)-1-associated phosphatidylinositol 3-kinase (29). Similar studies in rats have confirmed these findings and have demonstrated a decrease in IRS-1 tyrosine phosphorylation and an increase in protein kinase C $\theta$  activity, suggesting that FFA-induced insulin resistance may be secondary to activation of a serine kinase. Consequently, serine then phosphorylates IRS-1, which leads to reduced IRS-1 tyrosine phosphorylation (30). The present data are consistent with the hypothesis that troglitazone (and possibly all thiazolidinedione agents) improves insulin responsiveness via binding to PPAR- $\gamma$  receptors in the adipocyte, where PPAR- $\gamma$  receptors are mostly expressed, and that it promotes storage of FFAs in adipocyte triglycerides, which results in a reduction of intramyocellular triglyceride and fatty acylCoA concentrations. Consequently, the activation of protein kinase C $\theta$  and possibly other serine kinases would be prevented and would thereby reduce the degree of FFA-induced insulin resistance in skeletal muscle. Whether troglitazone and the other thiazolidinedione agents work mostly through this indirect mechanism, as opposed to a direct effect on muscle (as suggested by the in vitro data), to improve muscle insulin sensitivity remains to be determined.

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