

Troglitazone but not Metformin Restores Insulin-Stimulated Phosphoinositide 3-Kinase Activity and Increases p110 β Protein Levels in Skeletal Muscle of Type 2 Diabetic Subjects

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Insulin stimulation of phosphatidylinositol (PI) 3-kinase activity is defective in skeletal muscle of type 2 diabetic individuals. We studied the impact of antidiabetic therapy on this defect in type 2 diabetic subjects who failed glyburide treatment by the addition of troglitazone (600 mg/day) or metformin (2,550 mg/day) therapy for 3–4 months. Improvement in glycemic control was similar for the two groups, as indicated by changes in fasting glucose and HbA_{1c} levels. Insulin action on whole-body glucose disposal rate (GDR) was determined before and after treatment using the hyperinsulinemic (300 mU · m⁻² · min⁻¹) euglycemic (5.0–5.5 mmol/l) clamp technique. Needle biopsies of vastus lateralis muscle were obtained before and after each 3-h insulin infusion. Troglitazone treatment resulted in a 35 ± 9% improvement in GDR ($P < 0.01$), which was greater than ($P < 0.05$) the 22 ± 13% increase ($P < 0.05$) after metformin treatment. Neither treatment had any effect on basal insulin receptor substrate-1 (IRS-1)-associated PI 3-kinase activity in muscle. However, insulin stimulation of PI 3-kinase activity was augmented nearly threefold after troglitazone treatment (from 67 ± 22% stimulation over basal pre-treatment to 211 ± 62% post-treatment, $P < 0.05$), whereas metformin had no effect. The troglitazone effect on PI 3-kinase activity was associated with a 46 ± 22% increase ($P < 0.05$) in the amount of the p110 β catalytic subunit of PI 3-kinase. Insulin-stimulated Akt activity also increased after troglitazone treatment (from 32 ± 8 to 107 ± 32% stimulation, $P < 0.05$) but was unchanged after metformin therapy. Protein expression of other key insulin signaling molecules (IRS-1, the p85 subunit of PI 3-kinase, and Akt) was unaltered after either treatment. We conclude that the mechanism for the insulin-sensitizing effect of troglitazone, but not

metformin, involves enhanced PI 3-kinase pathway activation in skeletal muscle of obese type 2 diabetic subjects. *Diabetes* 51:443–448, 2002

Resistance to the effects of insulin on glucose uptake and metabolism in skeletal muscle is a major contributor to the pathogenesis of insulin-resistant states, such as obesity and type 2 diabetes (1,2). Intense interest has focused on identifying the steps in the insulin-signaling network that are responsible for the stimulation of glucose transport and that might be defective in insulin-resistant states. Data indicate that activation of phosphatidylinositol (PI) 3-kinase is a necessary, albeit not sufficient, step for insulin-induced glucose transport (3–6). Defective stimulation of PI 3-kinase activity by insulin has recently been identified in skeletal muscle of type 2 diabetic subjects (7,8).

Thiazolidinediones (TZDs) are a new class of insulin-sensitizing agents being used for the treatment of type 2 diabetes (9). The molecular targets of these compounds are thought to include the nuclear receptor peroxisome proliferator-activator receptor- γ (PPAR- γ), which regulates the expression of numerous genes involved in glucose and lipid metabolism (10). Evidence suggests that TZDs ameliorate insulin resistance and improve insulin-stimulated glucose disposal in skeletal muscle of type 2 diabetic subjects (11), but their exact mechanism of action remains unclear.

Several lines of evidence support the notion that TZDs enhance insulin signaling. TZDs have been shown to increase insulin-induced PI 3-kinase and Akt activation in cultured human skeletal muscle cells in vitro (12), with parallel increases in insulin-stimulated glucose uptake and glycogen synthesis (12). In addition, TZDs can prevent and reverse hyperglycemia-induced insulin resistance of insulin receptor and insulin receptor substrate-1 (IRS-1) phosphorylation in fibroblasts (13). In vivo in obese Zucker rats, TZDs normalize the phosphorylation of the skeletal muscle insulin receptor (14). Taken together, these data suggest that the effects of TZDs to enhance insulin signaling could be important for their antidiabetic action. However, to date, the effect of TZDs on insulin signaling in vivo in insulin target tissues of humans has not been reported.

Metformin is a member of the biguanide class of com-

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GDR, glucose disposal rate; IRS-1, insulin receptor substrate-1; PI, phosphatidylinositol; PPAR- γ , peroxisome proliferator-activator receptor- γ ; TZD, thiazolidinedione.

pounds, which are also effective at lowering glucose concentrations in patients with type 2 diabetes (15). A number of studies have demonstrated that metformin inhibits gluconeogenesis, reduces hepatic glucose output, and lowers fasting blood glucose concentration (11,16). In addition to its effect on the liver, metformin has also been reported to decrease glucose concentrations by increasing peripheral insulin sensitivity and augmenting insulin-mediated glucose uptake in skeletal muscle of type 2 diabetic subjects (17). The precise mechanism for this action of metformin is incompletely understood, but in vitro studies indicate it could involve multiple effects, including increased translocation of GLUT1 and GLUT4 glucose transporters from intracellular vesicles to the cell surface (18) and increased binding of insulin to cell-surface insulin receptors (19). Whereas recent studies have shown that metformin can normalize insulin receptor tyrosine phosphorylation and PI 3-kinase and Akt activities in adipocytes exposed for long periods to high insulin levels in vitro (20), the effects of metformin on insulin signaling in human or rodent skeletal muscle are still unknown.

The present study was designed to determine whether the insulin-sensitizing effects of troglitazone or metformin could involve reversal of the defect in insulin-stimulated PI 3-kinase activation in muscle of obese type 2 diabetic subjects. In this study, we demonstrate that 3 months of troglitazone treatment in obese type 2 diabetic subjects significantly increases insulin-stimulated PI 3-kinase and Akt activities in skeletal muscle. However, this improvement in insulin signaling is not seen in subjects treated with metformin, despite similar improvement in blood glucose control. Our data suggest that the mechanism for the insulin-sensitizing effect of troglitazone, but not metformin, could involve enhanced PI 3-kinase pathway activation in skeletal muscle of obese type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS

Human subjects and treatment protocol

A total of 14 type 2 diabetic subjects (11 men and 3 women between the ages of 30 and 70 years) who were poorly controlled ($HbA_{1c} > 8.5\%$ and fasting plasma glucose > 140 mg/dl) on at least half-maximal doses of any sulfonylurea agent were recruited for these studies. Except for diabetes, the subjects were healthy and on no other medications known to influence glucose metabolism. After screening, their current sulfonylurea medication was discontinued and all subjects were uniformly started on glyburide at 10 mg b.i.d. for at least 4 weeks. Baseline studies were subsequently performed and subjects randomized to the addition of either troglitazone or metformin to glyburide therapy. Subjects were counseled at 2-week intervals to consume a weight-maintenance diet for the duration of the study. Over 4–6 weeks the troglitazone treatment was titrated up to 600 mg/day or metformin up to 2,550 mg/day as required to achieve glycemic goals. After 3–4 months of this additional therapy, patients were readmitted for repeat studies. These subjects were part of a larger study group comparing the effects of troglitazone and metformin therapy on multiple clinical parameters (21). With regard to clinical parameters and response to treatment, the current subjects were indistinguishable from the larger study group. The experimental protocol was approved by the Committee on Human Investigation, University of California, San Diego. Informed written consent was obtained from all subjects after explanation of the protocol.

Protocol

All subjects were admitted to the Special Diagnostic and Treatment Unit at the VA San Diego Healthcare System. To quantitate peripheral insulin action, all subjects underwent a 3-h hyperinsulinemic ($300 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) euglycemic (5.0 – 5.5 mmol/l) clamp after a 12-h overnight fast, as previously described (22). The glucose disposal rate (GDR) was determined during the last 30 min of the clamp. Percutaneous needle biopsies of vastus lateralis muscle were performed before and after 3 h of insulin infusion, as previously described

(22), and muscle tissue was immediately frozen in liquid nitrogen. Plasma glucose and insulin levels were determined before each biopsy.

Preparation of muscle lysates

A total of 50 mg of muscle was homogenized using a polytron at half-maximum speed for 1 min on ice in 500 μl buffer A (20 mmol/l Tris [pH 7.5], 5 mmol/l EDTA, 10 mmol/l $\text{Na}_2\text{P}_2\text{O}_7$, 100 mmol/l NaF, and 2 mmol/l Na_2VO_4) containing 1% NP-40, 1 mmol/l phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin, and 10 mg/ml leupeptin. Tissue lysates were solubilized by continuous stirring for 1 h at 4°C and centrifuged for 10 min at 14,000g. The supernatants were stored at -80°C until analysis.

Determination of PI 3-kinase and Akt activity

Muscle lysates (150 μg protein) were subjected to serial immunoprecipitations with 5 μl of a polyclonal IRS-1 antibody (1:100 dilution; gift from Dr. Morris White, Joslin Diabetes Center) coupled to protein A-sepharose beads (Sigma, St. Louis, MO) overnight, followed by 2 μg of a polyclonal Akt antibody that recognizes both Akt1 and Akt2 (Upstate Biotechnology, Lake Placid, NY) coupled to protein G-sepharose beads (Pharmacia Biotech, Piscataway, NJ) for 4 h. The immune complex was washed, and PI 3-kinase and Akt activities were determined as previously described (7). The effect of insulin to stimulate Akt activity tended to be lower than in our previous report (7), probably because of a different immunoprecipitation methodology. In our previous report, we performed immunoprecipitation for 4 h and obtained approximately a twofold insulin stimulation ($300 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ for 3 h) of Akt activity over the basal value. Because of limitations in the size of muscle samples in the present study, we carried out serial immunoprecipitations (see above), which could result in less activation of Akt in response to insulin.

Determination of IRS-1, p85, p110 β , and Akt1/2 protein amounts

A total of 10 μg tissue lysate protein per lane was resolved by SDS-PAGE (7.5 and 10% gels) and transferred to nitrocellulose membranes (Schleicher & Schuell). The membranes were blocked with 5% nonfat dry milk overnight at 4°C and incubated with the following antibodies for 3 h at room temperature: a polyclonal IRS-1 antibody (Upstate Biotechnology), a polyclonal antibody against the p85 α subunit or p110 β subunit of PI 3-kinase (Upstate Biotechnology), and a polyclonal Akt antibody (Upstate Biotechnology) in 1% nonfat dry milk. The membranes were washed and bands visualized as previously described (23).

Statistical analysis

Data are presented as the mean \pm SEM. Statistical analyses were performed using the Stat View program (Abacus Concepts, Berkeley, CA). Statistical significance was tested with the paired Student's *t* test when appropriate.

RESULTS

Clinical and metabolic characteristics of the subjects

The subjects in the troglitazone and metformin groups were matched for age and obesity. After 3 months of troglitazone treatment, BMI was slightly increased compared with before treatment ($P < 0.05$). Subjects on metformin for 3 months had no change in BMI. HbA_{1c} , glucose, and insulin concentrations were significantly reduced by troglitazone therapy (Table 1). Triglyceride and free fatty acid concentrations tended to be reduced after troglitazone treatment. Similar to the effects of troglitazone, metformin treatment significantly decreased HbA_{1c} , glucose, and insulin concentrations (Table 1). Triglycerides were lower ($P < 0.05$) after metformin treatment (Table 1). The GDR, as determined by a hyperinsulinemic-euglycemic clamp, was increased by 35% ($P < 0.01$) after troglitazone treatment and by 22% ($P < 0.05$) after metformin (Table 1). The change in GDR after troglitazone treatment was greater than that seen after metformin treatment ($P < 0.05$).

PI 3-kinase activity

Figure 1A presents IRS-1-associated PI 3-kinase activity in muscle from obese type 2 diabetic subjects before and after treatment with troglitazone. The basal activity of PI 3-kinase was not altered by treatment. Insulin stimulated PI 3-kinase activity by 1.5-fold over basal levels ($P < 0.01$) before treatment. After troglitazone treatment, insulin stimulation was increased nearly threefold. The absolute

TABLE 1
Clinical and metabolic characteristics of the subjects

	Troglitazone			Metformin		
	Pretreatment	Posttreatment	Change from Pretreatment (%)	Pretreatment	Posttreatment	Change from Pretreatment (%)
Age (years)	56 ± 2	—	—	55 ± 2	—	—
BMI (kg/m ²)	34.0 ± 1.8	35.4 ± 2.1*	4 ± 2*	35.4 ± 3.7	34.8 ± 3.6	-2 ± 1
HbA _{1c} (%)	8.9 ± 0.4	7.3 ± 0.5†	-18 ± 6†	9.2 ± 0.6	7.1 ± 0.2†	-20 ± 5†
Glucose (mmol/l)	10.3 ± 0.4	6.8 ± 0.7†	-33 ± 8†	12.5 ± 0.5	7.6 ± 0.4‡	-39 ± 2‡
Insulin (pmol/l)	40 ± 7	33 ± 7*	-18 ± 9*	37 ± 6	33 ± 6*	-14 ± 6*
Triglyceride (mg/dl)	183 ± 23	163 ± 30	-11 ± 12	194 ± 41	160 ± 35*	-17 ± 7*
FFA (mmol/l)	0.53 ± 0.06	0.43 ± 0.04	-16 ± 10	0.49 ± 0.09	0.41 ± 0.05	-4 ± 15
GDR (mg · kg ⁻¹ · min ⁻¹)	5.99 ± 0.78	7.77 ± 0.8	35 ± 9†	6.22 ± 0.8	7.49 ± 0.9*	22 ± 13*

Data are means ± SEM for 7–10 subjects per group. Glucose and insulin levels were measured after an overnight fast. * $P < 0.05$ vs. pretreatment; † $P < 0.01$ vs. pretreatment; ‡ $P < 0.001$ vs. pretreatment. FFA, free fatty acid.

insulin-stimulated PI 3-kinase activity in muscle after troglitazone treatment was 67% greater than the pretreatment value ($P < 0.05$) (Fig. 1A).

Metformin treatment tended to increase basal PI 3-kinase activity, but this effect did not reach statistical significance ($P < 0.09$) (Fig. 1B). Insulin stimulated PI 3-kinase activity 2.6-fold before metformin treatment but unlike troglitazone, metformin therapy resulted in no further increase in IRS-1-associated PI 3-kinase in muscle (Fig. 1B). Insulin stimulated PI 3-kinase activity 1.9-fold after metformin treatment compared with 3-fold after troglitazone treatment. The magnitude of the increase in PI 3-kinase activity after troglitazone treatment was greater than the increase of PI 3-kinase activation with metformin treatment ($P < 0.05$).

Akt activity

Troglitazone treatment also significantly increased insulin-stimulated Akt activity compared with pretreatment levels (Fig. 2A). Before treatment, 3 h of insulin infusion resulted in a 1.3-fold stimulation of Akt activity. After 3 months of troglitazone therapy, basal Akt activity was unchanged, but insulin now stimulated Akt activity in skeletal muscle by 1.9-fold. Insulin-stimulated Akt activity was increased by 53% ($P < 0.05$) after treatment with troglitazone compared with pretreatment (Fig. 2A).

Metformin treatment did not alter Akt activity in the basal or insulin-stimulated state (Fig. 2B). The small effect of insulin to stimulate Akt activity before metformin treatment was similar to that in the troglitazone group before treatment (NS). However, in contrast to troglitazone treatment, there was no enhancement of Akt activation in response to insulin after metformin treatment (Fig. 2B).

IRS-1, p85, p110 β , and Akt1/2 protein levels

The effects of treatment on the expression of key insulin-signaling molecules in skeletal muscle were determined by Western blotting (Fig. 3). Figure 3 shows representative results from two subjects. Table 2 displays the quantitation of the blots from each group. Neither troglitazone nor metformin treatment had any effect on the expression of IRS-1, p85, and Akt1/2. However, with troglitazone, but not metformin treatment, the amount of the p110 β catalytic subunit of PI 3-kinase was increased by $46 \pm 22\%$ compared with pretreatment ($P < 0.05$) (Table 2).

DISCUSSION

A growing body of evidence implicates early and intermediate steps in the insulin signaling pathway, including the insulin receptor, IRS-1, and PI 3-kinase as candidates for defects contributing to insulin resistance in skeletal muscle of diabetic rodents and humans (7,8,24,25). The present studies were carried out to determine whether the defect in PI 3-kinase activation observed in obese type 2 diabetic people could be reversed by therapy with the insulin-sensitizing agents troglitazone or metformin. The major finding of this study is that treatment with the TZD troglitazone ameliorates the impairment of insulin-stimulated PI 3-kinase activity and enhances insulin-stimulated Akt activity in skeletal muscle of obese type 2 diabetic subjects. Unlike troglitazone, the biguanide metformin does not have these effects. These data suggest that the insulin-sensitizing action of troglitazone, but not metformin, on whole body glucose disposal in obese type 2 diabetic subjects may be caused, in part, by enhanced PI 3-kinase activation in skeletal muscle.

In the present study, both troglitazone and metformin treatment were efficacious in reducing plasma glucose levels and increasing insulin-stimulated GDR (Table 1),

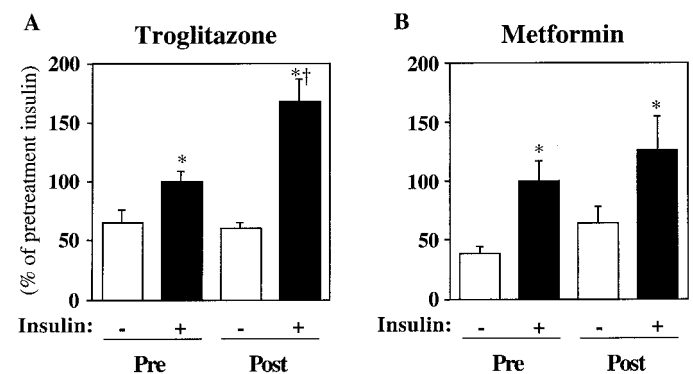


FIG 1. IRS-1-associated PI 3-kinase activity in skeletal muscle of diabetic subjects before and after troglitazone (A) or metformin (B) treatment. All subjects underwent a 3-h hyperinsulinemic-euglycemic clamp, and biopsies of vastus lateralis muscle were performed before and at the end of the clamp. PI 3-kinase activity was measured in IRS-1 immunoprecipitates and was quantitated using PhosphorImaging. Data are means ± SEM for 6–8 subjects per group. * $P < 0.05$ vs. no (-) insulin value for each group; † $P < 0.05$ for post- vs. pretreatment value for insulin-stimulated condition.

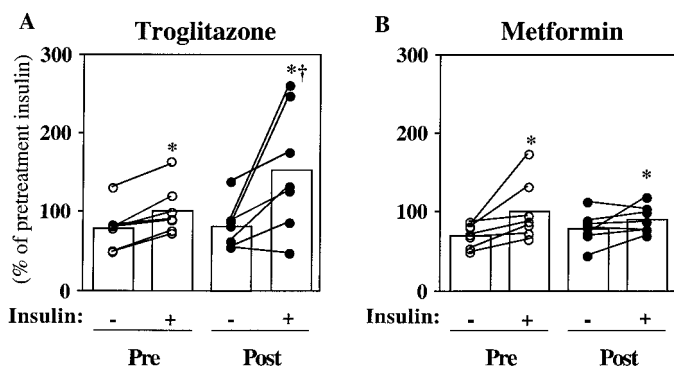


FIG 2. Akt1/2 kinase activity in skeletal muscle of diabetic subjects before and after troglitazone (A) or metformin (B) treatment. All subjects underwent a 3-h hyperinsulinemic-euglycemic clamp, and biopsies of vastus lateralis muscle were performed before and at the end of the clamp. Akt kinase activity was measured in muscle lysates that were subjected to immunoprecipitation with an antibody that recognizes both Akt1 and Akt2. The immunoprecipitated pellets were assayed for kinase activity using Crosstide as substrate (10). Each connected set of circles shows values from a single subject. Bars show mean for 6–8 subjects per group. * $P < 0.05$ vs. the no (–) insulin value for each group; † $P < 0.05$ for post- vs. pretreatment value for insulin-stimulated condition.

consistent with improved insulin sensitivity. Similar results for whole-body glucose homeostasis have been demonstrated in other studies of type 2 diabetic subjects and obese nondiabetic subjects (26,27), as well as in rodent models of diabetes and insulin resistance (28,29). The antidiabetic actions of troglitazone and metformin appear, however, to be mediated by different mechanisms. The principal action of troglitazone is to increase insulin-mediated peripheral glucose disposal in skeletal muscle, whereas metformin acts primarily by decreasing hepatic glucose output (11,16). Although many recent reports have

provided new insight into the mechanism of action of the insulin-sensitizing agents, only a few studies report the effects of TZDs or metformin on insulin action (12,13,21), and there are no data assessing the effects of TZDs or metformin on insulin signaling in skeletal muscle of humans in vivo.

A possible explanation for the enhancement of PI 3-kinase and Akt by troglitazone is that TZD activation of PPAR- γ directly or indirectly stimulates the activity of these two kinases in skeletal muscle through effects on gene expression. In cultured muscle cells from obese type 2 diabetic individuals, troglitazone exerts direct effects, increasing PPAR- γ mRNA and protein levels (30), together with improved insulin-stimulated glucose uptake and glycogen synthase activity (31). In addition, in vitro studies of human adipocytes demonstrate that PPAR- γ activation through TZD directly induces the expression of genes whose products are involved in insulin signaling (32, 33). In this study, we found that the amount of the p110 β catalytic subunit of PI 3-kinase was increased with troglitazone treatment, suggesting the improvement of PI 3-kinase activity could be caused, at least in part, by increased p110 β expression in skeletal muscle. Akt activity but not protein level in muscle is altered by TZD therapy, suggesting that the modulators of Akt activity may be under transcriptional control of PPAR- γ or that the increased PI3-kinase activity enhances insulin-stimulated Akt activity. Further data supporting the direct effect of TZDs on insulin signaling include the fact that TZD treatment of human skeletal muscle cells in vitro induces the activation of PI 3-kinase and Akt (12). Such effects could play an important role in the increased insulin-stimulated glucose uptake and glycogen synthesis that occurs in these TZD-treated cells (12,31).

In vivo and in vitro studies have demonstrated that chronic hyperglycemia can lead to the development of insulin resistance, which results from downregulation of glucose transport as well as alteration of insulin signaling in peripheral tissues (34–37). Restoration of euglycemia improves the impaired insulin stimulation of glucose transport in skeletal muscle of type 2 diabetic subjects (36) and the reduced Akt activity in skeletal muscle of diabetic rats (38). Based on these results, normalization of plasma glucose levels could be expected to lead to an improvement in the insulin-signaling cascade in type 2 diabetic subjects. Interestingly, we found that troglitazone but not metformin treatment significantly enhances insulin action at the level of PI 3-kinase and Akt in skeletal muscle. These differences occurred despite similar improvements in fasting glucose and HbA_{1c} concentrations with the two agents, thus making it unlikely that a generalized reduction in glycemia was responsible for changes in insulin signaling. Whether the degree of enhancement in insulin activation of PI 3-kinase and Akt in muscle after troglitazone treatment is sufficient to account for the increased whole-body glucose disposal beyond that attained with metformin treatment is not known.

Some of the effects of TZDs on insulin signaling may be through secondary mechanisms. Potential factors are plasma fatty acids and intramuscular lipids because elevations in these parameters are associated with insulin resistance (39,40). As previously reported (41,42), troglita-

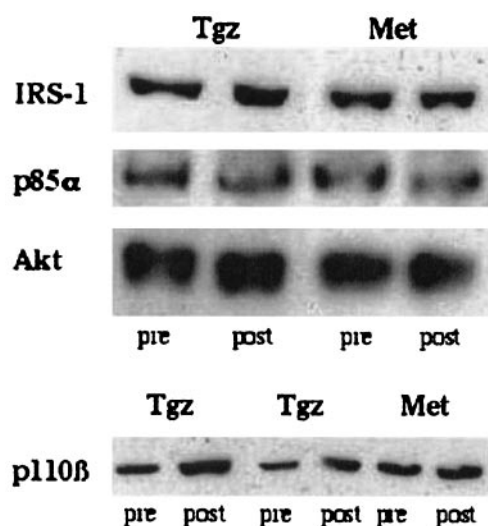


FIG 3. Effect of troglitazone or metformin treatment on insulin-signaling protein levels in skeletal muscle. Skeletal muscle was obtained before insulin infusion before (pre) or after (post) troglitazone or metformin treatment. Autoradiogram shows representative Western blots. For p110 β , blots for two troglitazone-treated subjects are presented to demonstrate the consistency of the effect. Quantitation of multiple blots on 7–8 subjects per treatment group is presented in Table 2.

TABLE 2
Expression levels of signaling proteins in skeletal muscle

	Troglitazone		Metformin	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
IRS-1	15.0 ± 4.7	12.8 ± 4.1	14.7 ± 3.9	12.3 ± 3.6
P85	10.1 ± 2.1	10.5 ± 2.3	10.1 ± 2.5	10.2 ± 2.1
P110β	4.2 ± 0.7	5.8 ± 0.9*	3.7 ± 0.4	3.1 ± 0.6
Akt1/2	17.1 ± 4.3	17.1 ± 3.0	11.0 ± 1.6	9.4 ± 1.7

Data are means ± SEM for 7–8 subjects per group. Proteins in muscle lysates (10 μg) were separated by SDS/PAGE on 7.5 or 10% gels and transferred to nitrocellulose membranes. IRS-1, p85, p110β and Akt1/2 were visualized by immunoblotting with a specific antibodies. All protein levels were quantitated using a densitometer yielding arbitrary units. **P* < 0.05 difference in pre- vs. posttroglitazone treatment values.

zone treatment tended to lower free fatty acid concentrations (Table 1). TZDs can also reduce accumulation of muscle triglycerides and diacylglycerol (43). Because activation of protein kinase C by elevated diacylglycerol levels in muscle impairs insulin signaling (44), decreases in plasma lipid concentrations with troglitazone treatment could lead to improved insulin signaling by reducing diacylglycerol in muscle. Troglitazone may also reduce intramyocellular lipid content via PPAR-γ-activated redirection of free fatty acids from skeletal muscle to storage in adipocyte triglyceride (45). Thus, the ability of troglitazone to improve insulin action in skeletal muscle could involve multiple actions in both adipose and muscle tissue. Indeed, the increased BMI in our subjects after 3 months of troglitazone treatment may be caused by the potent adipogenic effect of TZDs (46) or the effect of these agents to promote fluid retention with increased plasma volume (47). Despite increased BMI, these subjects have improved insulin action greater than that in metformin-treated subjects.

Although the main mechanism by which metformin treatment in type 2 diabetic subjects ameliorates insulin resistance is reducing hepatic glucose production, metformin also increases insulin-stimulated GDRs into skeletal muscle (48,49). In vitro studies demonstrate that high doses of metformin increase insulin-stimulated glucose transport in isolated skeletal muscle from type 2 diabetic subjects (50). In the current study, despite an increase in the insulin-stimulated glucose disposal rate with metformin therapy, metformin was unable to enhance insulin activation of PI 3-kinase and Akt in skeletal muscle. In addition, other reports have shown that the defect of glycogen synthase activity in obese type 2 diabetic subjects was not restored by metformin treatment (51). It is therefore likely that the molecular mechanism whereby metformin exerts its effects on glycemic control is mediated through other signaling pathways that appear to include activation of AMP kinase (52). This pathway could mediate the effect of metformin to redistribute glucose transporters from an intracellular compartment to the plasma membrane (53).

In summary, this is the first demonstration that the activities of the insulin-stimulated kinases PI 3-kinase and Akt in human skeletal muscle are regulated by therapy with insulin-sensitizing agents. Our data demonstrate that troglitazone treatment improves the impairment of PI 3-kinase activation and enhances Akt activation by insulin in skeletal muscle of obese type 2 diabetic subjects. The change in insulin-stimulated PI 3-kinase activity with tro-

glitazone treatment could be contributed to, in part, by increased expression of p110β. Although troglitazone is no longer available for the treatment of type 2 diabetes, the effects on insulin signaling in skeletal muscle are likely to be shared by the TZD class of compounds. In support of this, our preliminary data show that rosiglitazone treatment also increases insulin-stimulated PI 3-kinase and Akt in some insulin target tissues of insulin-resistant mice with adipose-specific GLUT4 knock out (data not shown). In contrast, treatment of diabetic humans with the biguanide metformin has no effect on PI 3-kinase or Akt activation in muscle. Thus, the insulin-sensitizing effect of the TZD troglitazone in obese type 2 diabetic subjects may be caused, at least in part, by enhancing the insulin-signaling cascade in skeletal muscle.

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REFERENCES

- White MF, Yenush L: The IRS-signaling system: a network of docking proteins that mediate insulin and cytokine action. *Curr Top Microbiol Immunol* 228:179–208, 1998
- DeFronzo RA: Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes. *Diabetes Rev* 5:177–269, 1997
- Cheatham B, Vlahos CJ, Cheatham L, Wang L, Blenis J, Kahn CR: Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. *Mol Cell Biol* 14:4902–4911, 1994
- Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M: Essential role of phosphatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes: studies with a selective inhibitor wortmannin. *J Biol Chem* 269:3568–3573, 1994
- Le Marchand-Brustel Y, Gautier N, Cormont M, Van Obberghen E: Wortmannin inhibits the action of insulin but not that of okadaic acid in skeletal muscle: comparison with fat cells. *Endocrinology* 136:3564–3570, 1995
- Quon MJ, Chen H, Ing BL, Liu ML, Zarnowski MJ, Yonezawa K, Kasuga M, Cushman SW, Taylor SI: Roles of 1-phosphatidylinositol 3-kinase and ras in regulating translocation of GLUT4 in transfected rat adipose cells. *Mol Cell Biol* 15:5403–5411, 1995

7. Kim YB, Nikoulina SE, Ciaraldi TP, Henry RR, Kahn BB: Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. *J Clin Invest* 104:733–741, 1999
8. Bjornholm M, Kawano Y, Lehtihet M, Zierath JR: Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation. *Diabetes* 46:524–527, 1997
9. Mudaliar S, Henry R: New oral therapies for type 2 diabetes mellitus: the gliitazones or insulin sensitizers. *Annu Rev Med* 52:239–257, 2001
10. Kersten S, Desvergne B, Wahli W: Roles of PPARs in health and disease. *Nature* 405:421–424, 2000
11. Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V, Shulman GI: Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med* 338:867–872, 1998
12. Peraldi P, Xu M, Spiegelman BM: Thiazolidinediones block tumor necrosis factor- α -induced inhibition of insulin signaling. *J Clin Invest* 100:1863–1869, 1997
13. Kellerer M, Kroder G, Tippmer S, Berti L, Kiehn R, Mosthaf L, Häring H: Troglitazone prevents glucose-induced insulin resistance of insulin receptor in rat-1 fibroblasts. *Diabetes* 43:447–453, 1994
14. Hevener AL, Reichart D, Olefsky J: Exercise and thiazolidinedione therapy normalize insulin action in the obese Zucker fatty rat. *Diabetes* 49:2154–2159, 2000
15. Bailey CJ, Turner RC: Metformin. *N Engl J Med* 334:574–579, 1996
16. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE: Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med* 333:550–554, 1995
17. Cusi K, DeFronzo RA: Metformin: a review of its metabolic effects. *Diabetes Rev* 6:89–131, 1998
18. Matthaei S, Hamann A, Klein HH, Benecke H, Kreyman G, Flier JS, Greten H: Association of metformin's effect to increase insulin-stimulated glucose transport with potentiation of insulin-induced translocation of glucose transporters from intracellular pool to plasma membrane in rat adipocytes. *Diabetes* 40:850–857, 1991
19. Fantus IG, Brosseau R: Mechanism of action of metformin: insulin receptor and postreceptor effects in vitro and in vivo. *J Clin Endocrinol Metab* 63:898–905, 1986
20. Pryor PR, Liu SC, Clark AE, Yang J, Holman GD, Tosh D: Chronic insulin effects on insulin signalling and GLUT4 endocytosis are reversed by metformin. *Biochem J* 348 Pt 1:83–91, 2000
21. Kim DD, Chu NV, Kong APS, Mudaliar SR, Henry RR, Baxi S, Armstrong DA: Glycemic effects of troglitazone versus metformin in poorly controlled type 2 diabetes mellitus patients (Abstract). *Diabetes* 49 (Suppl. 2):A113, 2000
22. Thorburn AW, Gumbiner B, Bulacan F, Wallace P, Henry RR: Intracellular glucose oxidation and glycogen synthase activity are reduced in non-insulin-dependent (type II) diabetes independent of impaired glucose uptake. *J Clin Invest* 85:522–529, 1990
23. Kim YB, Peroni OD, Franke TF, Kahn BB: Divergent regulation of Akt1 and Akt2 isoforms in insulin target tissues of obese Zucker rats. *Diabetes* 49:847–856, 2000
24. Folli F, Saad MJ, Backer JM, Kahn CR: Regulation of phosphatidylinositol 3-kinase activity in liver and muscle of animal models of insulin-resistant and insulin-deficient diabetes mellitus. *J Clin Invest* 92:1787–1794, 1993
25. Kerouz NJ, Horsch D, Pons S, Kahn CR: Differential regulation of insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. *J Clin Invest* 100:3164–3172, 1997
26. Iwamoto Y, Kuzuya T, Matsuda A, Awata T, Kumakura S, Inooka G, Shiraishi I: Effect of new oral antidiabetic agent CS-045 on glucose tolerance and insulin secretion in patients with NIDDM. *Diabetes Care* 14:1083–1089, 1991
27. Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N Engl J Med* 331:1188–1193, 1994
28. Fujiwara T, Yoshioka S, Yoshioka T, Ushiyama I, Horikoshi H: Characterization of new oral antidiabetic agent CS-045: studies in KK and ob/ob mice and Zucker fatty rats. *Diabetes* 37:1549–1558, 1988
29. Lee MK, Miles PD, Khoursheed M, Gao KM, Moossa AR, Olefsky JM: Metabolic effects of troglitazone on fructose-induced insulin resistance in the rat. *Diabetes* 43:1435–1439, 1994
30. Park KS, Ciaraldi TP, Lindgren K, Abrams-Carter L, Mudaliar S, Nikoulina SE, Tufari SR, Veerkamp JH, Vidal-Puig A, Henry RR: Troglitazone effects on gene expression in human skeletal muscle of type II diabetes involve up-regulation of peroxisome proliferator-activated receptor- γ . *J Clin Endocrinol Metab* 83:2830–2835, 1998
31. Park K-S, Ciaraldi TP, Abrams-Carter L, Mudaliar S, Nikoulina SE, Henry RR: Troglitazone regulation of glucose metabolism in human skeletal muscle cultures from obese type II diabetic subjects. *J Clin Endocrinol Metab* 83:1636–1643, 1998
32. Smith U, Gogg S, Johansson A, Olausson T, Rotter V, Svalstedt B: Thiazolidinediones (PPAR γ agonists) but not PPAR α agonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes. *FASEB J* 15:215–220, 2001
33. Rieusset J, Auwerx J, Vidal H: Regulation of gene expression by activation of the peroxisome proliferator-activated receptor γ with rosiglitazone (BRL 49653) in human adipocytes. *Biochem Biophys Res Commun* 265:265–271, 1999
34. Rossetti L, Smith D, Shulman GI, Papachristou D, DeFronzo RA: Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. *J Clin Invest* 79:1510–1515, 1987
35. Yki-Jarvinen H, Helve E, Koivisto VA: Hyperglycemia decreases glucose uptake in type I diabetes. *Diabetes* 36:892–896, 1987
36. Zierath JR, Galuska D, Nolte LA, Thorne A, Kristensen JS, Wallberg-Henriksson H: Effects of glycaemia on glucose transport in isolated skeletal muscle from patients with NIDDM: in vitro reversal of muscular insulin resistance. *Diabetologia* 37:270–277, 1994
37. Pillay TS, Xiao S, Olefsky JM: Glucose-induced phosphorylation of the insulin receptor: functional effects and characterization of phosphorylation sites. *J Clin Invest* 97:613–620, 1996
38. Krook A, Kawano Y, Song XM, Efendic S, Roth RA, Wallberg-Henriksson H, Zierath JR: Improved glucose tolerance restores insulin-stimulated Akt kinase activity and glucose transport in skeletal muscle from diabetic Goto-Kakizaki rats. *Diabetes* 46:2110–2114, 1997
39. Jucker BM, Rennings AJ, Cline GW, Shulman GI: ^{13}C and ^{31}P NMR studies on the effects of increased plasma free fatty acids on intramuscular glucose metabolism in the awake rat. *J Biol Chem* 272:10464–10473, 1997
40. Shulman GI: Cellular mechanisms of insulin resistance. *J Clin Invest* 106:171–176, 2000
41. Antonucci T, Whitcomb R, McLain R, Lockwood D, Norris RM: Impaired glucose tolerance is normalized by treatment with the thiazolidinedione troglitazone. *Diabetes Care* 20:188–193, 1997
42. Petersen KF, Krssak M, Inzucchi S, Cline GW, Dufour S, Shulman GI: Mechanism of troglitazone action in type 2 diabetes. *Diabetes* 49:827–831, 2000
43. Schmitz-Peiffer C, Oakes ND, Browne CL, Kraegen EW, Biden TJ: Reversal of chronic alterations of skeletal muscle protein kinase C from fat-fed rats by BRL-49653. *Am J Physiol* 273:E915–E921, 1997
44. Lewis RE, Volle DJ, Sanderson SD: Phorbol ester stimulates phosphorylation on serine 1327 of the human insulin receptor. *J Biol Chem* 269:26259–26266, 1994
45. Auwerx J: PPAR γ , the ultimate thrifty gene. *Diabetologia* 42:1033–1049, 1999
46. Spiegelman BM: PPAR- γ : adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47:507–514, 1998
47. Saleh YM, Mudaliar SR, Henry RR: Metabolic and vascular effects of the thiazolidinedione troglitazone. *Diabetes Rev* 7:55–76, 1999
48. Prager R, Scherthaner G, Graf H: Effect of metformin on peripheral insulin sensitivity in non insulin dependent diabetes mellitus. *Diabetologia* 12:346–350, 1986
49. Hother-Nielsen O, Schmitz O, Andersen PH, Beck-Nielsen H, Pedersen O: Metformin improves peripheral but not hepatic insulin action in obese patients with type II diabetes. *Acta Endocrinol (Copenh)* 120:257–265, 1989
50. Galuska D, Nolte LA, Zierath JR, Wallberg-Henriksson H: Effect of metformin on insulin-stimulated glucose transport in isolated skeletal muscle obtained from patients with NIDDM. *Diabetologia* 37:826–832, 1994
51. Damsbo P, Hermann LS, Vaag A, Hother-Nielsen O, Beck-Nielsen H: Irreversibility of the defect in glycogen synthase activity in skeletal muscle from obese patients with NIDDM treated with diet and metformin. *Diabetes Care* 21:1489–1494, 1998
52. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Diebber T, Fuji N, Musi N, Hirshman MF, Goodyear LJ, Moller DE: Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108:1167–1174, 2001
53. Hundal HS, Ramlal T, Reyes R, Leiter LA, Klip A: Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. *Endocrinology* 131:1165–1173, 1992