Thiazolidinediones (TZDs) are believed to induce insulin sensitization by modulating gene expression via agonistic stimulation of the nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ). We have shown earlier that the TZD troglitazone inhibits mitochondrial fuel oxidation in isolated rat skeletal muscle. In the present study, rat soleus muscle strips were exposed to TZDs to examine whether the inhibition of fuel oxidation is mediated by PPAR-γ activation. Our findings consistently indicated direct, acute, and PPAR-γ-independent TZD action on skeletal muscle fuel metabolism. Rapid stimulation of lactate release by 20 μmol/l troglitazone within 30 min suggested that direct TZD action on skeletal muscle in vitro does not rely on changes in gene expression rates (12.6 ± 0.6 [control] vs. 16.0 ± 0.8 μmol · g⁻¹ · h⁻¹ [troglitazone]; P < 0.01). This conclusion was supported by the failure of actinomycin D and cycloheximide to block the effects of troglitazone. Mitochondrial fuel oxidation was consistently inhibited by six different TZDs (percent inhibition of CO₂ production from palmitate after 25 h: troglitazone, 61 ± 2%; pioglitazone, 43 ± 7%; rosiglitazone, 22 ± 6%; BM13.1258, 47 ± 9%; BM15.2054, 51 ± 4%; and T-174, 59 ± 4% [P < 0.005 each]), but not by PPAR-γ agonistic compounds not belonging to the TZD class (JTT-501, 5 ± 7% [NS]; prostaglandin J₂, 17 ± 7% [P < 0.05]), which further argues against dependence on PPAR-γ activation. In summary, our findings provided good evidence that direct inhibition of mitochondrial fuel oxidation in isolated skeletal muscle is a group-specific effect of TZDs and is independent of PPAR-γ-mediated changes in gene expression. *Diabetes* 50:2309–2315, 2001

**Insulin resistance is a common metabolic abnormality associated with obesity, hypertension, and type 2 diabetes (1). Thiazolidinediones (TZDs) are a class of oral antidiabetic agents that improve insulin sensitivity and glucose homeostasis in type 2 diabetic patients (2–4) as well as in various animal models of diabetes and obesity (2–9). The TZDs troglitazone, rosiglitazone, and pioglitazone have already been used in clinical practice, but the mechanisms by which TZDs improve insulin sensitivity as well as deranged glucose and lipid metabolism are not yet fully understood (2–4,10).**

TZDs are agonistic ligands of peroxisome proliferator-activated receptor-γ (PPAR-γ), which belongs to the nuclear hormone receptor superfamily of transcription factors (5,6,10–12). Upon stimulation, PPAR-γ binds to responsive elements located in the promoter regions of many genes and modulates their transcriptional activities (2,10). Convincing evidence for an important role of PPAR-γ in TZD-induced insulin sensitization includes insulin sensitization by PPAR-γ agonists that do not belong to the TZD class (13–16) and by LG-100.268, an agonist of retinoid X receptor (RXR), which is the heterodimeric partner of PPAR-γ (17). Furthermore, a strong correlation of antidiabetic efficacies of PPAR-γ agonists in vivo with their respective potentials to bind and activate PPAR-γ in vitro has been demonstrated (6,12,16).

Although there is evidence to support PPAR-γ having an important role in TZD-induced insulin sensitization, TZDs also seem to address other mechanisms that do not involve PPAR-γ. That metabolic responses to TZDs in several experimental setups are independent of PPAR-γ-induced gene transcription is indicated by their rapid occurrence (5,7,18–24) and by a failure of the amplitudes of such responses to reflect the PPAR-γ-activating efficacies of different TZDs (5,20). The relevance of PPAR-γ is specifically unclear in skeletal muscle, which quantitatively is the most important target tissue for insulin and plays a predominant role in TZD-induced improvement of glucose homeostasis (25,26). Although PPAR-γ mRNA is abundant in fat and hardly found in skeletal muscle (10,27,28), the abundance of PPAR-γ protein in muscle was recently reported to be 67% of that in fat (29). However, clear experimental evidence for any functional
PPAR-γ signaling in skeletal muscle has never been provided. Because relevance of direct TZD effects on skeletal muscle for antidiabetic action is still being debated, we examined the interaction of TZDs with isolated skeletal muscle fuel metabolism.

We recently reported that troglitazone had rapid and direct action on fuel metabolism of freshly isolated rat skeletal muscle in vitro, action that was characterized by distinct inhibition of CO₂ production from palmitate and glucose (19). Troglitazone shifted glycolytic flux from the aerobic toward the anaerobic pathway simultaneous with glycogen depletion, which was marked after exposure of muscle specimens to troglitazone for 25 h (19). In the present study, we aimed to investigate the mechanisms responsible for direct interaction of TZDs with in vitro fuel handling in skeletal muscle using specimens of isolated rat soleus muscle. In particular, the studies were designed to provide evidence for or against the hypothesis that TZDs directly affects muscle fuel metabolism independent of PPAR-γ-induced modulation of gene expression.

**RESEARCH DESIGN AND METHODS**

**Rats.** Male SD rats were purchased from the breeding facilities of the University of Vienna (Himberg, Austria) and were used at a body weight of ~140 g. Obese Zucker rats (HadIII: fa/fa) were obtained from Harlan-Winkelmann (Borchern, Germany) and were used at age ~5 months (body weight ~750 g). Rats were kept in an artificial 12 h light/dark cycle at constant room temperature. Conventional laboratory diet and tap water were provided ad libitum until the evening before rats were killed, when only food was withdrawn. Rats were killed by cervical dislocation between 8:30 and 9:30 A.M.

All experiments were performed according to local law and the principles of good laboratory animal care.

**Compounds.** The PPAR-γ agonists TZDs troglitazone, pioglitazone, and rosiglitazone, as well as the RXR agonist LG-100.268 (17), were generously provided by Boehringer-Mannheim/LaRoche (Mannheim, Germany); the TZD T-174 (8) was generously provided by Tanabe (Saitama, Japan); the non-TZD PPAR-γ ligands JTT-501, prostaglandin J₂, LG-100.268, and vitamin E succinate were generously provided by Sankyo (Tokyo, Japan); the TZDs BM13.1258 and BM15.2054 (5) were generously provided by Winkelmann (Borchen, Germany) and were used at age 5 months (body weight 750 g). Rats were kept in an artificial 12 h light/dark cycle at constant room temperature. Conventional laboratory diet and tap water were provided ad libitum until the evening before rats were killed, when only food was withdrawn. Rats were killed by cervical dislocation between 8:30 and 9:30 A.M. All experiments were performed according to local law and the principles of good laboratory animal care.

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**Long-term muscle incubation (5 or 25 h).** Immediately after rats were killed, two (SD rats) or three (Zucker rats) longitudinal soleus muscle strips per leg (i.e., four or six strips per rat) were prepared, weighed (~25 mg/strip), and tied under tension on stainless steel clips, as previously described (31).

According to procedures used earlier (19,32), muscles were immediately put into 50-ml Erlenmeyer flasks coated with BlueSlick solution (Serva, Heidelberg, Germany) and placed into a shaking water bath (four or six strips per flask, 37°C, 130 cycles/min). Each flask contained 20 ml Cell Culture Medium 199 (5.5 mmol/l glucose [pH 7.35]; cat. no. M-4530; Sigma, St. Louis, MO) supplemented with 0.3% (wt/vol) fatty acid–free bovine serum albumin (BSA), 5 mmol/l HEPES, 25,000 units/l penicillin G, and 25 mg/l streptomycin. Palmitate was dissolved in ethanol and added to the medium to give final concentrations of 300 nmol/l palmitate and 0.5% ethanol (vol/vol). An atmosphere of 95% O₂/5% CO₂ was continuously provided within the flasks.

Immediately after preparation (one strip per ask). Each flask contained a continuous atmosphere of 95% O₂/5% CO₂ and 3 ml Krebs-Ringer buffer solution (pH 7.35) supplemented with 5.5 mmol/l glucose, 0.3% (wt/vol) BSA, 300 nmol/l palmitate, 0.5% (vol/vol) ethanol, and 0.1% (vol/vol) DMSO. In short-term muscle experiments, the pretreatment period lasted for 30 min, after which muscles were immediately transferred into another set of flasks.

Then muscles were incubated for another 30 min in 3 ml of identical buffer solution, which was supplemented with the above-described radioactive tracers and 30 mmol/l insulin (measurement period). A total of 20 μmol/l troglitazone was added to the incubation medium during the measurement period only or during both pretreatment and measurement periods, resulting in troglitazone exposure periods of 30 and 60 min, respectively. Finally, muscles were quickly removed, blotted, frozen, and lysed in KOH for further analytical procedures.

**Analytical procedures.** Net uptake of [U-14C]glucose or [U-14C]palmitate into glycogen was determined using [U-14C]glucose or H₂[¹⁴C]palmitate into [¹⁴C]glycogen, as previously described (31). Rates of CO₂ production were calculated from the conversion of [¹⁴C]glucose or [¹⁴C]palmitate into [¹⁴C]CO₂, which was trapped with a solution containing methanol and phenylmethylamine (1:1) (33). Rates of lactate release were calculated from the amount of lactate accumulated in the incubation medium during the measurement period; this concentration was determined enzymatically using the lactate dehydrogenase method (34).

For the determination of muscle glycogen content prevailing at the end of the experiment, glycogen in the muscle homogenate was completely degraded to glucose-6-phosphate and glyceraldehyde-3-phosphate (33). Glucose was then measured enzymatically by a commercial kit (Human, Tausnusser, Germany).

**Positive control experiments on cycloheximide and actinomycin D.** To confirm that cycloheximide efficiently blocked protein synthesis in our experimental setup, muscle strips were incubated in the absence or presence of 1 mg/l cycloheximide under the described conditions, except that culture medium devoid of methionine was used (modified Eagle’s medium; cat. no. 31900-020, Life Technologies, Paisley, U.K.), which was supplemented with all ingredients as listed above for Medium 199 plus 0.25 μmol/l [³⁵S]methionine. The effects of troglitazone and cycloheximide on soleus muscle fuel handling were not influenced by the medium used (Medium 199 versus modified Eagle’s medium; data not shown). After 4 or 24 h, muscles were quickly removed, blotted, and frozen for the determination of net [³⁵S]methionine incorporated into protein. Later, muscle specimens were thawed and immediately lysed in 0.5 ml NaOH (1 mol/l), and the protein was precipitated with 0.6 mol/l perchloric acid (1 mol/l). After centrifugation, the supernatant was discarded and the pellet was redissolved in 0.5 ml NaOH. After this procedure was repeated twice, 0.6 mol perchloric acid were added and the sample was counted for [³⁵S]radioactivity.

To provide evidence that 1 mg/l actinomycin D inhibited transcription in our experimental setup, muscle strips were homogenized immediately after incubation for 24 h in Medium 199, as described above. Total RNA was extracted from muscle homogenates with RNAiso B following the instructions of the manufacturer (Tel-Test, Friendswood, TX), and the RNA content of the extracts was determined photometrically (ratio 260:280 nm >1.8).

**Statistics.** All results are given as means ± SE. P values were calculated by two-tailed paired Student’s t test, and P < 0.05 was considered significant.
TABLE 1
Rates of insulin-stimulated (100 nmol/l) fuel metabolism in soleus muscle strips from SD rats exposed to 0 (control) or 5 μmol/l troglitazone for 25 h

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Control</th>
<th>Troglitazone</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ production from palmitate</td>
<td>nmol palmitate · g⁻¹ · h⁻¹</td>
<td>60</td>
<td>76 ± 3</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>CO₂ production from glucose</td>
<td>nmol glucose · g⁻¹ · h⁻¹</td>
<td>56</td>
<td>1,920 ± 66</td>
<td>727 ± 24</td>
</tr>
<tr>
<td>Lactate release</td>
<td>μmol · g⁻¹ · h⁻¹</td>
<td>70</td>
<td>24.4 ± 0.5</td>
<td>52.6 ± 1.0</td>
</tr>
<tr>
<td>Glucose transport</td>
<td>cpm · mg⁻¹ · h⁻¹</td>
<td>70</td>
<td>366 ± 32</td>
<td>1,333 ± 28</td>
</tr>
<tr>
<td>Glycogen synthesis</td>
<td>μmol glucose · g⁻¹ · h⁻¹</td>
<td>58</td>
<td>2.30 ± 0.10</td>
<td>1.30 ± 0.04</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>μmol glucosyl units/g</td>
<td>58</td>
<td>12.1 ± 0.4</td>
<td>9.1 ± 0.3</td>
</tr>
</tbody>
</table>

Data are means ± SE.

RESULTS

Effects of 25-h troglitazone exposure. In agreement with our previous report (19), the exposure of rat soleus muscle strips to 5 μmol/l troglitazone for 25 h distinctly modulated insulin-stimulated fuel metabolism (Table 1). Rates of CO₂ production from both palmitate and glucose were inhibited by ~62%, whereas lactate release more than doubled (+116%). In parallel, [³H]2DG transport was enhanced by +34%, and the rate of net glucose incorporation into glycogen was decreased by ~43%. Compared with that of control specimens, the glycogen content of muscle strips was ~25% lower after incubation with troglitazone (all troglitazone effects, P < 0.0001) (Table 1).

In parallel to the reduced conversion of extracellular substrates to CO₂ (Table 1), troglitazone inhibited CO₂ production from prelabeled intracellular substrate stores under basal conditions (troglitazone-induced change as percent of control; CO₂ from palmitate, −33 ± 4%, n = 6, P < 0.0001; CO₂ from glucose, −40 ± 6%, n = 6, P < 0.001) and after stimulation with 100 nmol/l insulin (CO₂ from palmitate, −45 ± 6%, n = 5, P < 0.001; CO₂ from glucose, −40 ± 10%, n = 5, P < 0.005).

Time dependence of troglitazone action. Exposure of SD rat muscle to 20 μmol/l troglitazone for 30 min significantly increased the rate of insulin-stimulated lactate release (+27%; P < 0.01). After 60 min, the troglitazone-induced increase in lactate release was accompanied by significant reductions in mitochondrial fuel oxidation and glycogen storage (Table 2).

Interaction of troglitazone with cycloheximide and actinomycin D. Positive control experiments confirmed that cycloheximide and actinomycin D acted as inhibitors of protein synthesis and transcription, respectively, in our specific experimental setup. Cycloheximide blocked protein synthesis—that is, [³⁵S]methionine incorporation into protein—in isolated rat soleus muscle to 17 ± 3 and 13 ± 2% of control over 4 and 24 h, respectively (P < 0.0001 each; n = 6 each). After exposure to actinomycin D for 24 h, the amount of total RNA extractable from soleus muscle strips was reduced by 28% (0.87 ± 0.06 vs. 1.20 ± 0.17 mg/g wet wt; n = 12; P < 0.05), indicating inhibition of transcriptional activity.

Marked effects of both cycloheximide and actinomycin D on fuel handling of isolated rat muscle were observed after 25 h, whereas only minor effects of actinomycin D but no effects of cycloheximide were observed after 5 h. All results were consistent, in that they clearly demonstrated the failure of cycloheximide and actinomycin D to block troglitazone’s inhibitory action on fuel oxidation and glycogen synthesis. Thus, the troglitazone-induced reductions in glycogenesis or CO₂ production from palmitate and glucose were not affected by inhibition of gene expression (Fig. 1).

Troglitazone action on insulin-resistant muscle. Following the same protocol used for muscle strips obtained from SD rats (Table 1), the dosage-dependent effects of troglitazone exposure in vitro were tested in soleus muscle strips obtained from genetically obese Zucker rats (fa/fa), which exhibit severe insulin resistance (31). Using concentrations of 0.63–10 μmol/l troglitazone, a dose-dependent inhibition of fuel conversion to CO₂ was observed in association with increased anaerobic glycolysis and glycogen depletion (Fig. 2). At 5 μmol/l troglitazone, CO₂ production from palmitate and glucose changed ~48% and

TABLE 2
Rates of insulin-stimulated (30 nmol/l) fuel metabolism in soleus muscle strips from SD rats exposed to 0 (control) or 20 μmol/l troglitazone for 30 or 60 min

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>min</th>
<th>Control</th>
<th>Troglitazone</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ production from palmitate</td>
<td>nmol palmitate · g⁻¹ · h⁻¹</td>
<td>30</td>
<td>109 ± 9</td>
<td>105 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>114 ± 6</td>
<td>90 ± 6</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>CO₂ production from glucose</td>
<td>nmol glucose · g⁻¹ · h⁻¹</td>
<td>30</td>
<td>213 ± 8</td>
<td>208 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>237 ± 13</td>
<td>201 ± 16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lactate release</td>
<td>μmol · g⁻¹ · h⁻¹</td>
<td>30</td>
<td>12.6 ± 0.6</td>
<td>16.0 ± 0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>14.2 ± 0.6</td>
<td>17.4 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose transport</td>
<td>cpm · g⁻¹ · h⁻¹</td>
<td>30</td>
<td>675 ± 35</td>
<td>678 ± 44</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>664 ± 24</td>
<td>653 ± 45</td>
<td>NS</td>
</tr>
<tr>
<td>Glycogen synthesis</td>
<td>μmol glucose · g⁻¹ · h⁻¹</td>
<td>30</td>
<td>6.03 ± 0.30</td>
<td>5.60 ± 0.30</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>6.57 ± 0.45</td>
<td>5.23 ± 0.36</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Glycogen content</td>
<td>μmol glucosyl units/g</td>
<td>30</td>
<td>14.2 ± 0.6</td>
<td>13.4 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>15.0 ± 0.7</td>
<td>13.2 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 12 each. NS, nonsignificant.

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-66%, respectively; lactate release, +234%; glucose transport, +110%; glycogen synthesis, -27%; and glycogen content, -64%.

**Comparison of troglitazone to other compounds.** Muscle specimens were exposed for 25 h to 5 μmol/l of the various agents listed in Fig. 3, which depicts the percent of metabolic rates found in intraindividual control muscle, which was incubated in the absence of any drug (absolute rates for control and troglitazone-exposed muscle strips given in Table 1).

All six PPAR-γ agonistic TZDs tested shifted fuel utilization from aerobic toward anaerobic pathways, as indicated by blunted rates of CO₂ production from palmitate (at least \( P < 0.005 \)) and by increased rates of lactate release (\( P < 0.0001 \) each). Quantitative efficacies of the respective TZDs, however, varied considerably. Troglitazone triggered a very distinct response, whereas the potent PPAR-γ agonist rosiglitazone was revealed to be the weakest TZD in this setup; significant decreases in glucose oxidation (at least \( P < 0.001 \)), glycogen synthesis (at least \( P < 0.001 \)), and glycogen content (at least \( P < 0.05 \)) were obvious for most TZDs, but not for rosiglitazone.

Regarding the non-TZD PPAR-γ agonists prostaglandin J₂ and JTT-501, 5 μmol/l of these substances did not modulate fuel metabolism in the manner observed in response to TZDs. JTT-501 failed to affect any parameter

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**Figure 1.** Interactions of troglitazone with cycloheximide (A) and actinomycin D (B) on skeletal muscle fuel metabolism. Soleus muscle strips from SD rats were exposed to 1 mg/l cycloheximide or 1 mg/l actinomycin D for 5 or 25 h, and the effects of concomitant exposure to troglitazone (■; 10 μmol/l for 5 h or 5 μmol/l for 25 h) or no exposure to troglitazone (□) were determined. During the last hour of the incubation, insulin-stimulated (100 nmol/l) rates of glycogen synthesis and CO₂ production from palmitate and glucose were measured. Data are means ± SE.

**Figure 2.** Insulin-stimulated (100 nmol/l) fuel metabolism in soleus muscle strips from obese Zucker rats (fa/fa) age 5 months exposed to various concentrations of troglitazone for 25 h (■). Data are means ± SE. \( *P < 0.05, \quad \dagger P < 0.02, \quad \ddagger P < 0.01, \quad \S P < 0.005, \quad \|$ P < 0.001, \quad \¶ P < 0.0001 \) versus without troglitazone (□).
measured, and prostaglandin J₂ moderately stimulated glycogenesis and palmitate oxidation to CO₂ \( (P < 0.05) \) each.

Parallel experiments were performed using LG-100.268 and vitamin E succinate, which are not ligands for PPAR-\( \gamma \). Both compounds failed to influence glucose transport or glycogen metabolism. LG-100.268 inhibited CO₂ production \( (P < 0.0001) \) and marginally increased lactate release \( (P < 0.05) \), and vitamin E moderately decreased CO₂ production from both palmitate and glucose \( (P < 0.05) \) each.

**DISCUSSION**

There is evidence available to indicate that the antidiabetic action of TZDs in vivo relies on delayed and PPAR-\( \gamma \)-dependent mechanisms, and that TZDs may indirectly act on skeletal muscle via PPAR-\( \gamma \) activation in adipose tissue \( (2,11,35) \). However, the potential role and mechanism of direct and acute interaction of TZDs with skeletal muscle are still unclear. We demonstrated in an earlier study that troglitazone distinctly affects fuel handling of isolated rat soleus muscle strips in vitro. Thus troglitazone rapidly shifts fuel utilization from aerobic toward anaerobic pathways, increases the rate of glucose transport, and inhibits glycogen storage \( (19) \). The present study extended our previous investigations by demonstrating that CO₂ production is reduced not only from extracellular, but also from intracellular, substrates. Furthermore, we demonstrated that other TZDs share troglitazone’s ability to directly interact with isolated rat skeletal muscle, and have provided clear evidence that this interaction is not based on the common property of TZDs to modulate gene transcription and protein synthesis via the activation of PPAR-\( \gamma \).

First, the experiments on time dependence and interaction with actinomycin D and cycloheximide argue against any role for PPAR-\( \gamma \)-mediated transcription or translation in troglitazone’s direct effects on muscle fuel metabolism in vitro. Thus distinct responses to troglitazone occurred within 30 min and became marked within 60 min, a finding that would argue against the established view that triggering metabolic effects via the modulation of gene expression requires a more prolonged time period. In addition, actinomycin D and cycloheximide, currently regarded as potent blockers of gene transcription and protein synthesis, respectively, clearly failed to counteract troglitazone action in our experimental setup.

Second, our experiments revealed that direct troglitazone action on isolated skeletal muscle is independent of concomitant insulin stimulation \( (\text{see also } 19) \), and that muscles isolated from obese Zucker rats, which exhibit lower rates of glucose metabolism than muscles from lean littermates \( (31) \), had similar dose-dependent responses to troglitazone as did muscle strips from normally insulin-sensitive SD rats \( (\text{detailed dose-response curve for SD rat muscle in } 19) \). These findings indicate major differences between TZD in vivo versus in vitro action, because the metabolic effects of TZDs in vivo are seen only in the presence of insulin, and TZD action is hardly detectable in healthy rodents but is very distinct in insulin-resistant rodents.

**FIG. 3.** Rates of insulin-stimulated (100 nmol/l) fuel metabolism in soleus muscle strips from SD rats exposed for 25 h to 5 μmol/l each of the following: the PPAR-\( \gamma \) agonistic TZDs troglitazone, pioglitazone, rosiglitazone, BM15.1258, BM15.2054, and T-174 \( (\blacksquare) \); the non-TZD PPAR-\( \gamma \) agonists prostaglandin J₂ and JTT 501 \( (\boxed{\text{●}}) \); and the RXR agonist LG-100.268 \( (\boxed{\text{□}}) \); and vitamin E succinate \( (\boxed{\text{□}}) \). Data are given as percent of an intraindividual control value as determined in the absence of the respective compound. Data are means ± SE. \( *P < 0.05, \dagger P < 0.01, \ddagger P < 0.005, \§ P < 0.002, \| P < 0.001, \|\| P < 0.0001 \) versus control.
animals (7,9). Furthermore, the inhibition of fuel oxidation observed in obese rat muscle exposed to TZDs in vitro contrasts with the increased glucose oxidation and insulin sensitization of the glycogenic pathway prevailing in skeletal muscle isolated from insulin-resistant rodents orally treated with TZDs in vivo (5,36,37). Given the widely accepted assumption that TZD-induced insulin sensitization in vivo relies on PPAR-γ activation (2,6,10–12,16), such divergences in TZD in vivo versus in vitro action support our conclusion that a different—i.e., a PPAR-γ-independent—mechanism must underlie modulation of fuel metabolism in isolated muscle.

Third, we compared the efficacies of different PPAR-γ agonistic compounds and found further evidence against the involvement of PPAR-γ. Our results clearly indicated that the observed shift in fuel utilization from aerobic toward anaerobic pathways is specific for drugs belonging to the TZD class. This conclusion is based on the finding that all six TZDs tested markedly stimulated lactate re- 

toxication, however, is reversed by these TZDs.

tivity, in which case troglitazone is ranked higher than pioglitazone. The former differences in ef- 
cacies among the TZDs used are likely to 

cially mechanisms of action. The contribution of 

dependent actions to the bene- 
ication being an early and essential step in TZD-induced 
independent action. The contribution of 

dependent mechanisms of action. The contribution of 

clyric and Metabolism.

ACKNOWLEDGMENTS

\[\text{REFERENCES}\]

1. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and athero- 

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We thank Sankyo (Tokyo, Japan), Tobacco (Osaka, Japan), Boehringer-Mannheim/LaRoche (Mannheim, Germany), and Tanabe (Saitama, Japan) for generously providing the compounds. We also thank the staff at the Biomedical Research Center, University of Vienna, for taking care of the rats.

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