Normalization of Skeletal Muscle Glycogen Synthesis and Glycolysis in Rosiglitazone-Treated Zucker Fatty Rats

An In Vivo Nuclear Magnetic Resonance Study

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The aim of this study was to characterize insulin-stimulated skeletal muscle glucose metabolism in Zucker fatty rats and to provide insight into the therapeutic mechanism by which rosiglitazone increases insulin-stimulated glucose disposal in these rats. Metabolic parameters were measured using combined in vivo 13C nuclear magnetic resonance (NMR) spectroscopy to measure skeletal muscle glucose uptake and its distributed fluxes (glycogen synthesis and glycolysis), and 31P NMR was used to measure simultaneous changes in glucose-6-phosphate (G-6-P) during a euglycemic-hyperinsulinemic clamp in awake Zucker fatty rats. Three groups of Zucker fatty rats (fatty rosiglitazone [FRSG], fatty control [FC], lean control [LC]) were treated for 7 days before the experiment (3 mg/kg rosiglitazone or vehicle via oral gavage). Rates of glycolysis and glycogen synthesis were assessed after treatment by monitoring 1,6-13C2 glucose label incorporation into 1-13C glycogen, 3-13C lactate, and 3-13C alanine during a euglycemic (7–8 mmol/L)-hyperinsulinemic (10 mU/L) clamp. The FRSG group exhibited a significant increase in insulin sensitivity, reflected by an increased whole-body glucose disposal rate during the clamp (24.4 ± 1.9 vs. 17.6 ± 1.4 and 33.2 ± 2.0 mg kg⁻¹ min⁻¹ in FRSG vs. FC [P < 0.05] and LC [P < 0.01] groups, respectively). The increased insulin-stimulated glucose disposal in the FRSG group was associated with a normalization of the glycolytic flux (52.9 ± 9.1) to LC (56.2 ± 16.6) versus FC (18.8 ± 8.6 nmol·g⁻¹·min⁻¹, P < 0.02) and glycogen synthesis flux (56.3 ± 11.5) to LC (75.2 ± 15.3) versus FC (16.6 ± 12.8 nmol·g⁻¹·min⁻¹, P < 0.05). [G-6-P] increased in the FRSG and LC groups versus baseline during the clamp (13.0 ± 11.1 and 16.9 ± 5.8%, respectively), whereas [G-6-P] in the FC group decreased (−23.3 ± 13.4%, P < 0.05). There were no differences between groups in intramyocellular glucose, as measured by biochemical assay. These data suggest that the increased insulin-stimulated glucose disposal in muscle after rosiglitazone treatment can be attributed to a normalization of glucose transport and metabolism. Diabetes 51:2066–2073, 2002

Rosiglitazone, a member of the thiazolidinedione (TZD) class of oral antihyperglycemic agents used to treat type 2 diabetes, is a potent peroxisome proliferator–activated receptor (PPAR-γ) agonist. Studies in animal models of type 2 diabetes show that rosiglitazone improves glycemic control by enhancing insulin-stimulated whole-body glucose disposal (1,2). The increase in whole-body insulin sensitivity is the result of increased insulin action in skeletal muscle (1,3,4), liver (1,4), and adipose tissue (5,6). Skeletal muscle accounts for the largest proportion of insulin-stimulated whole-body glucose uptake (7); therefore, much of the focus on the insulin-sensitizing benefits of TZDs has been targeted to this tissue (1,3,4,8–12). Although skeletal muscle expresses low levels of PPAR-γ (13) and direct actions of TZDs on muscle glucose metabolism in vitro have been reported (5,9,14), the precise mechanism of action of TZDs in skeletal muscle is unclear. Much evidence exists to suggest that the improvement in muscle insulin sensitivity may be an indirect consequence of activation of PPAR-γ in fat, a tissue in which the receptor is abundantly expressed. Activation of PPAR-γ in fat results in decreased adipocyte lipolysis and hence free fatty acid availability (1,2,4,15). This in turn would lead to a subsequent decrease in intramyocellular lipid content (4). A number of recent clinical studies have demonstrated the direct correlation between intramyocellular lipid content and insulin sensitivity (16–18). It is also well established that an increase in plasma free fatty acids results in decreased insulin-stimulated glucose uptake in skeletal muscle via an inhibition of the insulin-signaling cascade (19,20) and/or inhibition of...
glycogen synthesis during substrate competition (glucose–fatty acid cycle) (21).

However, a comprehensive analysis of the effects of TZDs on muscle glycogen uptake and its subsequent metabolism in vivo has not been performed. The purpose of the present study was to use a combination of in vivo $^{13}$C and $^{31}$P nuclear magnetic resonance (NMR) noninvasive spectroscopic techniques to simultaneously measure skeletal muscle glycogen uptake, glycolysis, glycogenolysis, and glucose-6-phosphate (G-6-P) under euglycemic-hyperinsulinemic clamp conditions in conscious Zucker fatty rats following rosiglitazone treatment.

**RESEARCH DESIGN AND METHODS**

**Animals.** Zucker obese (fa/fa) rats and their lean littermates (Harlan, Indianapolis, IN) were housed in an environmentally controlled room with a 12-h light/dark cycle. Obese and nonobese rats (8–10 weeks old) were placed on a pair-fed diet ($\#5L35$; LabDiet, Richmond, IN) consisting of 100 kcal/day – 40% fructose, 10% sucrose, 50% casein. Rats were placed into three groups: Zucker obese (fa/fa) rats; Zucker lean (fa/−) rats; and nonobese lean (fa/−) rats. Prior to the day of the experiment, rats were anesthetized with sodium pentobarbital (Abbott Laboratories, Chicago) so they could be placed in the restraining tube. A euglycemic–hyperinsulinemic clamp was begun using 1,6- $^{13}$C$_2$ glucose (99% enriched, 20% $^{13}$C in the sodium adduct of normal glucose, and m/z = 205 ion corresponding to the sodium adduct of 1,6- $^{13}$C$_2$ glucose. Standard curves were generated (0–97% 1,6- $^{13}$C$_2$ glucose, atom percent excess [APE]). Plasma samples were extracted using ice-cold acetone, and 5 μl was injected into the column.

Glycogen 1,6- $^{13}$C$_2$ fractional enrichments were determined using the isolated glycogen after perchloric acid extraction (21), and absolute glycogen concentrations were measured using a separate portion of muscle as previously described (24). Intramyocellular glycogen was calculated by measuring free glycogen in the tissue extracts and correcting for extracellular space using the turnover of the plasma glucose pool must be faster than that of the lactate column.

**Glycogen synthesis rate ($V_{GDR}$) calculation.** The incremental change in C-1 glycogen peak intensity from 1,6- $^{13}$C$_2$ glucose incorporation was measured at 100.5 ppm. Incremental plasma glucose $^{13}$C fractional enrichment as well as final glycogen $^{13}$C enrichment and concentrations were used to back-extrapolate the glycogen concentration (μmol/g, which represents micromoles glycol units per gram muscle wet weight) at each measured time point to baseline, as described by Bloch et al. (26). Glycogen synthesis rates were determined using a linear regression analysis over the individual time point glycogen concentrations.

**Glycolytic flux calculations.** Metabolic steady-state conditions were assumed for calculating carbon flux through the glycolytic pathway into the intermediate triose pool of lactate, pyruvate, and alanine (Fig. 1). Differential equations were developed from steady-state rate equations and solved for glycolytic flux ($V_{GP}$) (27). $^{13}$C label incorporation from 1,6- $^{13}$C$_2$ glucose into $^{3}$C lactate and $^{3}$C alanine in the hindlimb muscles may be used as an indirect marker of pyruvate labeling. Therefore, the $^{3}$C lactate and $^{3}$C alanine turnover rates provide a qualitative index of $V_{GP}$. The absolute flux was calculated by simultaneously solving the differential equations describing the $V_{GP}$ using CWave software (Dr. Graeme F. Mason, Yale University). The parameters required for the modeling of $V_{GP}$ include plasma 1,6- $^{13}$C$_2$ glucose, tissue 3- $^{13}$C lactate and 3- $^{13}$C alanine turnover rates and lactate and alanine concentrations. A limitation of this measurement is that the turnover of the plasma glucose pool must be faster than that of the lactate and alanine pools. Therefore, these measurements can only be made under hyperinsulinemic conditions when plasma glucose turnover is increased (27).

The traditional method by which skeletal muscle $V_{GP}$ is measured in vivo is an indirect one. It is calculated as the difference between glucose uptake rates as assessed using the 2-deoxyglucose method and glycolysis synthesis rate as

**Tissue state calculations.** Plasmid tissue extracts were prepared for high-field NMR analysis by homogenizing ~0.5 g skeletal muscle as previously described (21). NMR analysis was performed at 0.4 Tesla (Bruker WB-400 AMX NMR spectrometer; Bruker). Proton Observed carbon-enhanced spectroscopy was performed on tissue extract samples for fractional enrichment calculations (21). For quantification, a correction factor was calculated when a TR (pulse repetition time) equaling 30 s was used. Alanine concentration was quantified by comparing its signal intensity with a known internal concentration standard (lactate), which was measured in tissue extracts using a 2300 STAT PLUS analyzer (Yellow Springs Instruments, Yellow Springs, OH).

**Analytical procedures.** Plasma glucose concentrations were measured using a 2300 STAT PLUS analyzer. Plasma immunoactive free insulin was measured with a double-antibody radioimmunoassay technique (Linco Research, St. Charles, MO). The 1,6- $^{13}$C$_2$ enrichment of plasma glucose was determined using a LCMS (liquid chromatography/mass spectrometry) system (Agilent Technologies LC/MSD 1100) (23). The MS electrospray chamber conditions were set for positive ionization. Selected ion monitoring was used to detect the sodium adducts $^{23}$ (23) of each glucose isotope, with a m/z = 203 ion corresponding to the sodium adduct of normal glucose and a m/z = 205 ion corresponding to the sodium adduct of 1,6- $^{13}$C$_2$ glucose. Standard curves were generated (0–97% 1,6- $^{13}$C$_2$ glucose, atom percent excess [APE]). Plasma samples were extracted using ice-cold acetone, and 5 μl was injected into the column.

Glycogen 1,6- $^{13}$C$_2$ fractional enrichments were determined using the isolated glycogen after perchloric acid extraction (21), and absolute glycogen concentrations were measured using a separate portion of muscle as previously described (24). Intramyocellular glycogen was calculated by measuring free glycogen in the tissue extracts and correcting for extracellular space using an intra/extracellular space ratio of 9.0 and plasma glucose assumed to be equal to the extracellular space glucose (25). Plasma triglyceride concentrations were determined enzymatically using an Olympus AU600 analyzer (Olympus Optical, Melville, NY).

**Endogenous glucose production calculation.** Endogenous glucose production (EGP) was estimated by taking the difference of the glucose infusion rate ($G_{inf}$) and the tracer-determined whole-body glucose disposal rate (GDR). Whole-body GDR was calculated from $G_{inf}$ and the steady-state plasma glucose concentration with respect to the $^{13}$C enrichment before peak

**Field strength magnet.** Bruker Medical, Billerica, MA). Both $^{13}$C and $^{31}$P NMR spectroscopy were performed on a Bruker ABR system (horizontal/40-cm diameter bore, 4.7-Tesla field strength magnet, Bruker Medical, Billerica, MA). Both $^{13}$C and $^{31}$P NMR spectroscopy were performed using a triple tune radio frequency probe consisting of concentric surface coils (the outer $^{1}$H coil [42 mm] tuned to 200.21 MHz, and the inner dual frequency $^{13}$C coil [25 mm] tuned to 50.34 and 81.05 MHz, respectively). The rat hindlimb was positioned over the $^{13}$C/$^{31}$P coil (vertical in plane) and placed in the magnet isocenter. Because of the intrinsic low $^{13}$C/$^{31}$P sensitivity in the hindlimb experiments, it was necessary to measure the bulk signal from the larger tissue beds of mixed fiber type including the gastrocnemius and biceps femoris muscles. Rats were killed with a lethal dose of Nembutal (Abbott Laboratories). Superficial skin was rapidly removed from the left hindquarter followed by in situ freeze clamping of the gastrocnemius and biceps femoris muscles. Rats were anesthetized with an intravenous dose of 50 mg/kg Nembutal (Abbott Laboratories). Superficial skin was rapidly removed from the left hindquarter followed by in situ freeze clamping of the gastrocnemius and biceps femoris muscles. Rats were killed with a lethal dose of Nembutal.

**In vivo NMR spectroscopy.** All in vivo $^{13}$C and $^{31}$P NMR experiments were performed on a Bruker ABR system (horizontal/40-cm diameter bore, 4.7-Tesla field strength magnet, Bruker Medical, Billerica, MA). Both $^{13}$C and $^{31}$P NMR spectroscopy were performed using a triple tune radio frequency probe consisting of concentric surface coils (the outer $^{1}$H coil [42 mm] tuned to 200.21 MHz, and the inner dual frequency $^{13}$C or $^{31}$P coil [25 mm] tuned to 50.34 and 81.05 MHz, respectively). The rat hindlimb was positioned over the $^{13}$C/P coil (vertical in plane) and placed in the magnet isocenter. Because of the intrinsic low $^{13}$C/$^{31}$P sensitivity in the hindlimb experiments, it was necessary to measure the bulk signal from the larger tissue beds of mixed fiber type including the gastrocnemius and biceps femoris. $^{1}$H decoupled- $^{13}$C NMR spectroscopy was performed in the following manner: an initial frequency selective sinc pulse (20 ms) set on the low-field side of the methylene carbon of lipids at 30 ppm was immediately followed by a nonspecific sinc pulse set on the high-field side of the methylene carbon of lipids for 4 ms, and the sinc pulse power was adjusted to eliminate most of the signals in that region. Broadband $^{1}$H Waltz-8 decoupling was applied during acquisition, and additional Nuclear Overhauser Enhancement was achieved using low-power decoupling during the relaxation delay (TR = 0.5 s, SW = 10 KHz, 2 K data). A 15-min baseline spectrum was followed by subsequent 15-min acquisitions throughout the duration of the experiment. $^{13}$C NMR spectra were processed sequentially using a Gaussian filter and baseline flattening before peak integration (Nuts NMR processing software; Acorn NMR, Fremont, CA).

$^{31}$P NMR was performed using a hard pulse (45° flip angle) that was optimized 7 mm from the surface coil (TR = 1 s, SW = 3 K, 2 K data). Two 10-min spectra were acquired: one at baseline and the other at 140–150 min.
assessed using 3H or 14C glucose (28). Whole-body glycolysis may be calculated using the "H glucose washout method (29).

Statistical analysis. All data are reported as means ± SE. ANOVA was performed on data to determine the significance at a minimum threshold of \( P < 0.05 \) between the three groups. A multiple comparison Newman-Keuls post hoc test was used when necessary to determine significance between the groups.

RESULTS

Posttreatment baseline measurements. As a result of the pair-feeding protocol, there were no differences in weight between the FC (350 ± 4 g) and FRSG (345 ± 5 g) groups. However, both groups weighed significantly more than the LC group (291 ± 3 g, \( P < 0.001 \)). The weight gain during the treatment period was 29 ± 3, 30 ± 2, and 11 ± 1 g in the FC, FRSG, and LC groups, respectively (\( P < 0.001 \) LC vs. FC and FRSG). Surprisingly, there were no differences in basal insulin concentrations between the FRSG (428 ± 77 pmol/l) and FC (475 ± 111 pmol/l) groups, but both groups had significantly higher concentrations than the LC group (125 ± 28 pmol/l, \( P < 0.05 \)). Basal plasma glucose in the FRSG group (8.7 ± 0.6 mmol/l) was significantly reduced compared with that of the FC group (13.5 ± 0.8 mmol/l, \( P < 0.001 \)) and was normalized to the LC group (7.5 ± 0.4 mmol/l). Basal plasma triglyceride concentration in the FRSG group (114 ± 12 mg/dl) was also lower than that in the FC group (273 ± 37 mg/dl, \( P < 0.001 \)) but higher than that in the LC group (32 ± 3 mg/dl, \( P < 0.01 \)).

Euglycemic-hyperinsulinemic clamp. During the euglycemic-hyperinsulinemic clamp experiment, plasma glucose concentrations were maintained at ~7–8 mmol/l (Table 1). Although steady-state insulin concentrations during the euglycemic-hyperinsulinemic clamp were similar in the FC and FRSG groups, they were significantly higher than those in the LC group (Table 1). Glucose turnover was increased in the FRSG group after treatment. This may be visualized in Fig. 2 because the plasma 1,6-13C2 glucose APE increased rapidly during the clamp in the LC and FRSG groups but was slower in the FC group. \( G_{inff} \) at 120 min was significantly higher in the FRSG group (20.3 ± 1.9 mg · kg\(^{-1}\) · min\(^{-1}\)) than in the FC group (13.8 ± 1.4 mg · kg\(^{-1}\) · min\(^{-1}\), \( P < 0.05 \)) but lower than in the LC group (30.1 ± 1.8 mg · kg\(^{-1}\) · min\(^{-1}\), \( P < 0.01 \); Table 1). EGP did not differ in the three groups; therefore, whole-body GDR was also higher in the FRSG group than in the FC group. However, whole-body GDR in the FRSG group was not normalized to the LC group (Table 1).

In vivo 13C NMR. Baseline subtracted 13C NMR spectra depicting 13C label turnover in a LC rat during a euglycemic-hyperinsulinemic clamp is shown in Fig. 3. The \( \beta \) and \( \alpha \) anomer peaks of 13C glucose appear at 96.8 and 93.0 ppm, respectively, and the large peak slightly downfield at 100.5 ppm corresponds to the C-1 glucosyl unit of the glycogen polymer. The C-6 glucosyl unit of the glycogen polymer resides at 61.4 ppm and is significantly larger than C-1 because of the overlapping 6-13C peak. 3-13C lactate and 3-13C alanine may also be observed at 21.0 and 13.5 ppm, respectively, and the large peak slightly downfield at 100.5 ppm corresponds to the C-1 glucosyl unit of the glycogen polymer. The C-6 glucosyl unit of the glycogen polymer resides at 61.4 ppm and is significantly larger than C-1 because of the overlapping 6-13C peak. 3-13C lactate and 3-13C alanine may also be observed at 21.0 and 16.9 ppm, respectively. The absolute steady-state \( V_{glyc} \) was significantly reduced in the FRSG group (16.6 ± 12.8 nmol · g\(^{-1}\) · min\(^{-1}\)) versus the LC group (75.2 ± 15.3 nmol · g\(^{-1}\) · min\(^{-1}\), \( P < 0.05 \)) but was normalized in the FRSG group (56.3 ± 11.5 nmol · g\(^{-1}\) · min\(^{-1}\), \( P < 0.05 \) vs. FC, Table 1). The best-fit label turnover curves to 3-13C pyruvate are shown in Fig. 4. Empirically, the 3-13C pyruvate turnover in the LC and FRSG groups is more rapid than in the FC group. This increased label turnover in the LC and FRSG groups versus the FC group may be used as a qualitative
**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma glucose (mmol/l)</th>
<th>Plasma insulin (pmol/l)</th>
<th>EGP (nmol · g⁻¹ · min⁻¹)</th>
<th>Whole-body GDR (ng · kg⁻¹ · min⁻¹)</th>
<th>Vᵢₘ (mmol · g⁻¹ · min⁻¹)</th>
<th>Vᵢₚ (mmol · g⁻¹ · min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td>6.7 ± 0.6</td>
<td>39.1 ± 1.8</td>
<td>3.7 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>16.8 ± 0.8</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>FC</td>
<td>8.1 ± 0.4</td>
<td>30.1 ± 1.8</td>
<td>3.8 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>13.8 ± 1.4</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>FRSG</td>
<td>7.5 ± 0.3</td>
<td>39.0 ± 1.9</td>
<td>3.7 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>20.3 ± 0.4</td>
<td>4.2 ± 0.4</td>
</tr>
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</table>

Data are means ± SE. Plasma glucose, PCr (assigned to 0 ppm), and α₃, β₃, and γ-ATP were not normalized in these groups. Whole-body GDR was calculated at 120 min after steady-state glucose turnover had been achieved. *p < 0.001 vs. LC; †p < 0.05 vs. LC and FRSG groups, Fig. 5B.}

**DISCUSSION**

The data reported here show that after 7-day rosiglitazone treatment in the Zucker fatty rat, insulin-stimulated whole-body glucose disposal was increased and muscle glycogen synthesis and glycolysis were normalized. Although TZDs, including rosiglitazone, have been shown to improve glycemic control in animal models of type 2 diabetes by reducing insulin resistance in muscle (1,3,4), liver (1,4), and adipose tissue (5,6), in vivo characterization of the glucose metabolic fate in these tissues has been difficult to achieve. In the present study, an in vivo NMR spectroscopic technique was used to noninvasively measure insulin-stimulated glucose disposal in the hindlimb muscles of an awake rat.

Using in vivo ¹³C NMR spectroscopy, glycogen synthesis was shown to be increased in skeletal muscle of either type 2 diabetic (12) or insulin-resistant obese (30) subjects treated with troglitazone. Although whole-body glucose oxidation as measured by indirect calorimetry was increased in the troglitazone-treated type 2 diabetic subjects, the skeletal muscle Vᵢₘ was not ascertained (12). The direct in vivo effect of acute troglitazone stimulation on glycolysis was measured in perfused Sprague-Dawley rat hindlimb (31). The investigators showed that in the presence of troglitazone alone (no exogenous insulin), O₂ consumption and lactate/pyruvate release were increased with no exogenous fatty acids present in the perfusate.
Therefore, it was postulated that both aerobic and nonaerobic glycolysis was stimulated by the TZD. However, these hindlimb perfusion experiments were not performed in chronically TZD-treated or insulin-resistant animal models.

The necessity for an in vivo noninvasive measurement of both glycogen synthesis and glycolysis becomes increasingly apparent in the presence of differing results with regard to the glucose metabolic fate as measured in isolated muscle strips after in vitro TZD incubation (32,33) or chronic oral TZD treatment (34–37). Fürnsinn et al. (32) showed that in vitro troglitazone incubation elicited a glucose catabolic response in excised Sprague-Dawley soleus muscle strips. Using U-14C glucose tracer techniques for flux measurements, both glycogen synthesis and glucose oxidation were decreased after insulin administration, whereas anaerobic glycolysis (measured as lactate release) increased to the extent that the anaerobic/aerobic glycolysis ratio was ~50:1. However, these data contrast with a number of studies that have examined the in vivo effects of chronic oral TZD treatment on skeletal muscle glucose metabolism (glycogen synthesis and glycolysis) in insulin-resistant rodent models (34–37). Although direct in vivo measurements of these fluxes were not performed, these studies nevertheless show that TZDs can act by various degrees to normalize both glycogen synthesis and glycolysis. After 4-day englitazone treatment in ob/ob mice, both insulin-stimulated glycogen synthesis and glycolysis were normalized, as assessed in incubated soleus muscle strips using 1-14C glucose to measure gly-

**FIG. 2.** 1,6-13C2 glucose enrichment turnover kinetics during the euglycemic-hyperinsulinemic clamp. Absolute 1,6-13C2 glucose (APE) in the LC (●), FRSG (▲), and FC (■) groups during a steady-state euglycemic-hyperinsulinemic clamp is shown. Data are means ± SE.

**FIG. 3.** In vivo 13C NMR spectra of 1,6-13C2 glucose label incorporation into 1-13C and 6-13C glycogen, 3-13C lactate, and 3-13C alanine. A series of 15-min baseline subtracted spectra acquired during a euglycemic-hyperinsulinemic clamp are shown. 1-13C glucose (β-anomer, 96.8 ppm, and α-anomer, 93.0 ppm), 1-13C glycogen (100.5 ppm), 6-13C glycogen (61.4 ppm), 3-13C lactate (21.0 ppm), and 3-13C alanine (16.9 ppm) are visible where indicated.
cogen synthesis and glucose oxidation and $3^{3}H$ glucose to measure glycolysis (34). An interesting result of this study was that basal glucose oxidation was increased after englitazone treatment and normalized to the lean group. More recently, Sreenan et al. (35) showed that the increased insulin-stimulated glucose disposal, as measured using 2-deoxyglucose in 6-week troglitazone-treated ZDF rat soleus muscle, was also due to an increase in both glycogen synthesis and glycolysis. Also of note was that troglitazone almost doubled the basal glucose oxidation flux in these rats. This result correlated with an enhancement of muscle pyruvate dehydrogenase activity under basal conditions in the study. Fürnsinn et al. (36) examined the effects of treatment with the TZDs BM13.1258 and BM15.2054 on glucose intermediary metabolism. In this study, insulin-stimulated glycogen synthesis, as measured in isolated soleus, was increased after 10-day oral treatment of either TZD in Zucker fatty rats, yet glucose transport and oxidation was only increased in the BM13.1258-treated rats. These results suggest that different TZDs exhibit different responses with regard to glycogen synthesis and glucose oxidation and therefore may control glucose metabolism via multiple mechanisms.

Further to the notion that TZDs may exhibit control of glucose disposal through multiple mechanisms is that TZDs appear not to exhibit the same degree of insulin sensitivity in all tissue. Although both glycogen synthesis and glucose metabolism in hindlimb skeletal muscle of Zucker fatty rats were normalized after 7-day rosiglitazone treatment in the present study, whole-body GDR was only partially normalized (73% of control). This discrepancy is most likely due to a noneffect of rosiglitazone on insulin-stimulated glucose disposal in other tissue (e.g., heart and adipose tissue) (1).

Measurements of $[G\text{-6-P}]$ along with glycogen synthesis and V$_\text{gly}$ can be used to provide an index of metabolic control (12). For example, as insulin stimulated hexokinase and glycogen synthase in the FC group, glucose transport could not keep up with the demand, which resulted in a decrease in $[G\text{-6-P}]$ versus the LC and FRSG groups. This hypothesis holds true if intracellular glucose were held constant or lower in the FC versus the LC and
FRSG groups. Although there were no significant differences between the groups, there was large variability in our intracellular glucose measurement. Therefore, it is difficult to delineate the role of glucose transport versus hexokinase in the control of glucose uptake in this model. Although there is as yet no direct evidence for an effect of rosiglitazone on GLUT4 expression or function in skeletal muscle, rosiglitazone corrects expression levels of the skeletal muscle proteins cellubrevin, VAMP-2, and syntaxin-4, which are known to be associated with GLUT4 vesicle trafficking in skeletal muscle of hyperglycemic ZDF rats (38).

In summary, a combined in vivo $^{13}$C and $^{31}$P NMR spectroscopic approach was used to measure skeletal muscle glucose uptake and its distributed fluxes (glycogen synthesis and glycolysis) and G-6-P (a key metabolic intermediate) during a euglycemic-hyperinsulinemic clamp in awake Zucker fatty rats. The data suggest that the increased insulin-stimulated glucose disposal in skeletal muscle associated with rosiglitazone treatment was attributed to a normalization of both glycogen synthesis and glycolysis.

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REFERENCES
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