Rosiglitazone Improves Downstream Insulin Receptor Signaling in Type 2 Diabetic Patients

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Thiazolidinediones (TZDs) improve glycemic control and insulin sensitivity in patients with type 2 diabetes. To determine whether the TZD-induced improvement in glycemic control is associated with enhanced insulin receptor signaling in skeletal muscle, 20 type 2 diabetic patients received a 75-g oral glucose tolerance test (OGTT) and euglycemic insulin (80 mU · m^{-2} · min^{-1}) clamp with [3-^{3}H]glucose/indirect calorimetry/vastus lateralis muscle biopsies before and after 16 weeks of rosiglitazone treatment. Six age-matched nondiabetic subjects served as control subjects. RSG improved fasting plasma glucose (185 ± 8 to 139 ± 5 mg/dl), mean plasma glucose during the OGTT (290 ± 9 to 225 ± 6 mg/dl), HbA$_{1c}$ (8.5 ± 0.3 to 7.1 ± 0.3%), insulin-mediated total-body glucose disposal (TGD) (6.9 ± 0.7 to 9.2 ± 0.8 mg · kg^{-1} fat-free mass · min^{-1}) (all $P < 0.001$), and decreased fasting plasma free fatty acid (FFA) (789 ± 59 to 656 ± 50 μEq/l) and mean FFA during the OGTT (644 ± 41 to 471 ± 35 μEq/l) (both $P < 0.01$). Before RSG treatment, insulin infusion did not significantly increase insulin receptor tyrosine phosphorylation (0.95 ± 0.10 to 1.08 ± 0.13 density units; NS) but had a small stimulatory effect on insulin receptor substrate (IRS)-1 tyrosine phosphorylation (1.05 ± 0.10 to 1.21 ± 0.12 density units; $P < 0.01$) and the association of p85 with IRS-1 (0.94 ± 0.06 to 1.08 ± 0.06 activity units; $P < 0.01$). RSG therapy had no effect on basal or insulin-stimulated insulin receptor tyrosine phosphorylation but increased insulin stimulation of IRS-1 tyrosine phosphorylation (1.13 ± 0.11 to 1.56 ± 0.17 density units; $P < 0.01$ vs. prerosiglitazone) and p85 association with IRS-1 (1.00 ± 0.06 to 1.27 ± 0.07 activity units; $P < 0.05$ vs. prerosiglitazone). In control and type 2 diabetic subjects, TGD/nonoxidative glucose disposal correlated positively with the insulin-stimulated increments in IRS-1 tyrosine phosphorylation ($r = 0.52$/$r = 0.57$, $P < 0.01$) and inversely with the plasma FFA concentration during the insulin clamp ($r = -0.55$/$r = -0.53$, $P < 0.01$). However, no significant association between plasma FFA concentrations during the insulin clamp and the increment in either IRS-1 tyrosine phosphorylation or the association of p85 with IRS-1 was observed. In conclusion, in type 2 diabetic patients, rosiglitazone treatment enhances downstream insulin receptor signaling in muscle and decreases plasma FFA concentration while improving glycemic control. *Diabetes* 52:1943–1950, 2003

I nsulin resistance in skeletal muscle, the tissue responsible for 70–80% of insulin-stimulated glucose disposal during euglycemic-hyperinsulinemic clamp studies, is a characteristic feature of individuals with type 2 diabetes (1–3). A number of intracellular defects in insulin action in muscle have been described, including impaired insulin receptor signal transduction (4,5), decreased glucose transport (6) and glucose phosphorylation (7), and diminished glycogen synthase activity (8).

The thiazolidinediones (TZDs) represent a new class of insulin-sensitizing agents that have proven effective in the treatment of patients with type 2 diabetes (9,10). TZDs initiate their action by binding to a specific nuclear receptor termed the peroxisome proliferator–activated receptor (PPAR)-γ (11,12). The binding affinity of TZDs to PPAR-γ closely parallels their in vivo antihyperglycemic potency (13). PPAR-γ receptors are found primarily in adipocytes (14), and their concentration in muscle, the tissue responsible for the majority of insulin-mediated glucose disposal (1–3), is low (14). Consistent with their tissue distribution, PPAR-γ activation causes preadipocytes to differentiate into mature fat cells and induces key enzymes involved in lipogenesis (11,15,16). Numerous clinical studies have demonstrated that treatment with TZDs causes a 20–30% reduction in the fasting plasma free fatty acid (FFA) concentration, which is paralleled by a 20–30% reduction in the fasting plasma glucose (FPG) concentration and HbA$_{1c}$ in type 2 diabetic patients (17–21). It is well established that elevated plasma FFA levels induce insulin resistance in muscle (22–24) by a variety of mechanisms including impaired insulin signal transduction (25,26), glucose phosphorylation and transport (25,27), and glycogen synthase (28). These observations suggest that the TZD-mediated improvement in FFA metabolism and reduction in plasma FFA levels may, in part, contribute to their beneficial effects on insulin-stimulated glucose disposal in the skeletal muscle. Only one recently published study (29) has examined the effect of any TZD on the insulin signal transduction system in humans. These authors demonstrated that troglitazone treatment for ~4 months improved total glucose disposal and insulin-stimulated phosphatidylinositol (PI) 3-kinase and Akt activity in muscle. However, the insulin infusion rate during the euglycemic-hyperinsulinemic clamp was in the high pharmacological range (300 mU

TABLE 1
Effect of rosiglitazone treatment on body composition, glycemic control, and plasma lipids levels in type 2 diabetic subjects

	Control subjects	Diabetic subjects		P
		Before	Rosiglitazone	
Body weight (kg)	81.1 ± 3.9	84.1 ± 3.2	86.5 ± 3.5	0.0008
BMI (kg/m^2)	27.3 ± 1.3	30.5 ± 1.1	31.4 ± 1.2	0.0006
Fat mass (kg)	23.0 ± 1.8	27.7 ± 2.0	30 ± 2.1	0.0002
HbA$_{1c}$ (%)	4.9 ± 0.1*	8.5 ± 0.3	7.1 ± 0.3	0.0001
Fasting plasma glucose (mg/dl)	94 ± 2*	185 ± 8	139 ± 5	<0.0001
Fasting plasma insulin (μU/ml)	6 ± 1*	16 ± 1	11 ± 1	0.002
Fasting C-peptide (ng/ml)	1.2 ± 0.3	1.4 ± 0.2	1.4 ± 0.3	NS
Fasting plasma FFA (μEq/l)	625 ± 54	789 ± 59	646 ± 49	0.001
Total cholesterol (mg/dl)	170 ± 12	177 ± 10	192 ± 8	0.08
LDL cholesterol (mg/dl)	102 ± 10	88 ± 8	112 ± 9	0.009
HDL cholesterol (mg/dl)	44 ± 3†	34 ± 2	38 ± 2	0.001
Triglycerides (mg/dl)	115 ± 28	275 ± 83	214 ± 53	0.09
75-g OGTT				
Mean plasma glucose (mg/dl)	129 ± 8*	290 ± 9	225 ± 6	<0.0001
Mean plasma insulin (μU/ml)	45 ± 5	32 ± 4	29 ± 4	NS
Mean plasma C-peptide (ng/ml)	5.3 ± 0.9*	2.5 ± 0.4	3.1 ± 0.3	NS
Mean plasma FFA (μEq/l)	398 ± 53*	644 ± 41	465 ± 34	<0.0001
Insulinogenic index (0–30 min)	0.90 ± 0.17*	0.17 ± 0.03	0.21 ± 0.03	NS
Insulinogenic index (0–120 min)	2.37 ± 1.31*	0.16 ± 0.03	0.25 ± 0.07	0.04

Data are means ± SE. Comparative data in nondiabetic control subjects also are shown. Insulinogenic index (0–30 min) = Δinsulin/Δglucose from 0 to 30 min during the OGTT. Insulinogenic index (0–120 min) = ΔAUC insulin/ΔAUC glucose from 0 to 120 min during the OGTT. *$P <$ 0.01, †$P <$ 0.05 control vs. diabetic subjects before rosiglitazone treatment.

$\cdot$ m^{-2} $\cdot$ min^{-1}) and produced plasma insulin concentrations that were very nonphysiological.

In the present study, we have evaluated the effect of rosiglitazone treatment in type 2 diabetic subjects on glucose tolerance, insulin secretion, plasma lipid and FFA levels, peripheral (muscle) sensitivity to insulin, and the insulin signal transduction system in muscle using euglycemic insulin clamp in combination with a vastus lateralis skeletal muscle biopsy. The insulin infusion rate (80 mU $\cdot$ m^{-2} $\cdot$ min^{-1}) was chosen to produce plasma insulin concentrations in the high physiological range.

RESEARCH DESIGN AND METHODS

Subjects. A total of 20 type 2 diabetic patients (11 men and 9 women, age 54 ± 3 years, BMI 30.5 ± 1.1 kg/m^2, 6 Caucasian and 14 Mexican-American, duration of diabetes 6 ± 1 years, HbA$_{1c}$ 8.5 ± 0.3%, FPG 185 ± 8 mg/dl) (Table 1) were recruited from the outpatient clinic of the Texas Diabetes Institute. Eleven subjects had been taking a stable dose of sulfonylurea drugs for at least 3 months before the study and remained on the same dose of sulfonylurea throughout the study period. Nine subjects were treated with diet alone. Patients who had previously received insulin, metformin, or a TZD were excluded. Entry criteria included age from 30 to 70 years, BMI <38 kg/m^2, and an FPG concentration between 140 and 260 mg/dl. All subjects were in good general health without evidence of cardiac, hepatic, renal, or other chronic diseases, as determined by medical history, physical examination, and routine blood chemistries. Subjects were not consuming any medications known to affect glucose metabolism, and none were performing any regular physical activity. In all subjects, body weight was stable for at least 3 months before study. Six healthy individuals (Table 1) served as control subjects. All subjects gave signed voluntary informed consent before their participation. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

Study design. Four weeks before the study, all subjects met with a dietitian and were instructed to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein. During this period, FPG was measured at weekly intervals and varied by <5% in each subject. Blood pressure was measured during each visit, and HbA$_{1c}$ and fasting plasma lipids were measured twice during this period. During the week before the start of rosiglitazone treatment, all subjects *1*) received a 75-g oral glucose tolerance test (OGTT), *2*) were measured for fat mass and fat-free mass (FFM) using an intravenous bolus of ^{3}H$_2$O, and *3*) received a euglycemic insulin clamp in

combination with [3-^{3}H]glucose and indirect calorimetry to examine peripheral (muscle) tissue sensitivity to insulin. The bolus injection of ^{3}H$_2$O was performed on the same day as the OGTT. All studies were carried out in the postabsorptive state at 8:00 A.M. after a 10- to 12-h overnight fast. Sulfonylurea-treated subjects did not take their sulfonylurea on the day of the study. After completion of these studies, subjects were started on rosiglitazone (8 mg/day) for 16 weeks. During the rosiglitazone treatment period, subjects returned to the Clinical Research Center of Texas Diabetes Institute at 8:00 A.M. every 2 weeks for measurement of FPG and body weight and to check compliance with the treatment regimen. Fasting plasma lipids (total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol) and HbA$_{1c}$ were measured monthly. During the last week of the treatment period, the OGTT, euglycemic insulin clamp, and body fat measurements were repeated.

OGTT. Baseline blood samples for determination of plasma glucose, FFA, insulin, and C-peptide concentrations were drawn at −30, −15, and 0 min. At time 0, subjects ingested 75 g glucose in 300 ml orange-flavored water, and plasma glucose, FFA, insulin, and C-peptide concentrations were measured at 15-min intervals for 2 h. At time 0, a 100-μCi bolus of ^{3}H$_2$O was given and the plasma tritiated water radioactivity was determined at 90, 105, and 120 min for calculation of FFM and fat mass as previously described (30).

Insulin clamp with skeletal muscle biopsy. Subjects returned to the Clinical Research Center of the Texas Diabetes Institute at 8:00 A.M. 3–7 days after the OGTT for the hyperinsulinemic-euglycemic clamp with vastus lateralis muscle biopsies. A catheter was placed in an antecubital vein for the infusion of all test substances. A second catheter was placed retrogradely into a hand vein, and the hand was placed in a heated box (60°C) for sampling of arterialized blood. A primed (25 μCi × FPG/100)-continuous (0.25 μCi/min) infusion of [3-^{3}H]glucose was begun 180 min before the start of insulin infusion in the diabetic subjects (at −120 min in control subjects) to allow for isotopic equilibration. At time −60 min, a percutaneous muscle biopsy was obtained with a Bergstrom cannula from the vastus lateralis muscle under local anesthesia (31). Muscle biopsy specimens were immediately blotted free of blood, frozen in liquid nitrogen, and stored under liquid nitrogen until processing. Baseline arterialized venous blood samples for determination of plasma [3-^{3}H]glucose radioactivity and plasma glucose, FFA, and insulin concentrations were drawn at −30, −20, −10, −5, and 0 min. At time 0 (11:00 A.M.), a prime-continuous infusion of human regular insulin (Novolin; Novo Nordisk Pharmaceuticals, Princeton, NJ) was started at a rate of 80 mU $\cdot$ min^{-1} $\cdot$ m^{-2} body surface area and continued for 4 h to allow insulin to more fully exert its stimulatory effect on tissue glucose disposal. A second percutaneous muscle biopsy was obtained from a site about 4 cm distal to the first site 30 min after the start of insulin infusion (i.e., 90 min after the initial biopsy). In preliminary studies, we have shown that insulin stimulation of insulin receptor and insulin receptor substrate (IRS)-1 tyrosine phosphoryla-
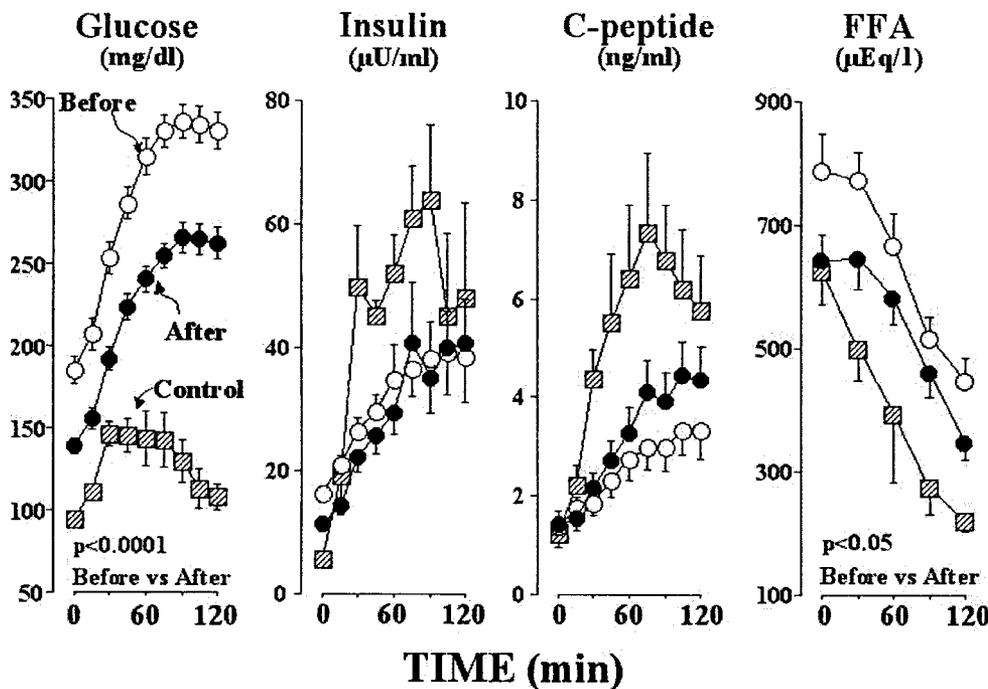
FIG. 1. Plasma glucose, insulin, C-peptide, and FFA concentrations in control subjects (▨) and type 2 diabetic patients during the 75-g OGTT before (○) and after (●) rosiglitazone treatment.

tion is maximum at 30 min (4). After initiation of the insulin infusion, the plasma glucose concentration in diabetic subjects was allowed to drop until it reached 100 mg/dl, at which level it was maintained by appropriately adjusting a variable infusion of 20% dextrose. In control subjects, the plasma glucose concentration was clamped at each subject's basal level. The insulin infusion was continued for 240 min to obtain a measure of insulin-mediated glucose disposal during the last 30 min of the insulin clamp (210- to 240-min time period). Continuous indirect calorimetry was performed with a ventilated hood system (Deltatrac II; SensorMedics, Yorba Linda, CA) during the last 40 min of the basal period and during the last 30 min of insulin infusion. Throughout the insulin clamp, blood samples for determination of plasma glucose concentration were drawn every 5 min. Blood samples for determination of plasma insulin and [3-^{3}H]glucose radioactivity were collected every 10–15 min, and blood samples for the determination of plasma FFA concentration were determined every 60 min.

Assays. Plasma glucose concentration was measured at bedside using the glucose oxidase method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). Plasma insulin (Diagnostic Products, Los Angeles, CA) and C-peptide (Diagnostic Systems Laboratories, Webster, TX) concentrations were measured by radioimmunoassay. HbA$_{1c}$ was measured by affinity chromatography (Biochemical Methodology, Drower 4350; Isolab, Akron, OH). Plasma FFA concentration was measured by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany). Plasma total cholesterol, HDL cholesterol, and triglyceride levels were measured enzymatically (Boehringer Mannheim, Indianapolis, IN) on a Hitachi 704 autoanalyzer. LDL cholesterol was calculated from the Friedewald equation. Tritiated glucose specific activity was determined in deproteinized plasma samples. Insulin receptor and IRS-1 tyrosine phosphorylation and the amount of p85 associated with IRS-1 were assayed using immunoprecipitation and immunoblot analysis, as previously described (4). The association of PI 3-kinase activity with IRS-1 was assayed by determining the ability of anti–IRS-1 immunoprecipitates to incorporate [^{32}P]ATP into PI (4).

Calculations. Under steady-state postabsorptive conditions, the rate of endogenous glucose appearance (R_a) is calculated as the [3-^{3}H]glucose infusion rate (dpm/min) divided by the steady-state plasma [3-^{3}H]glucose specific activity (dpm/mg). During the insulin clamp, nonsteady conditions prevailed, and R_a was calculated from Steele's equation (32). Endogenous glucose production (EGP) was calculated as the R_a minus the exogenous glucose infusion rate. Total-body glucose disposal (TGD) equals the sum of residual EGP plus the exogenous glucose infusion rate. Total-body glucose metabolic clearance rate (MCR) equals the TGD rate divided by the steady-state plasma glucose concentration, where TGD is expressed as milligrams per kilogram FFM per minute and plasma glucose is expressed as milligrams per milliliter. Rates of glucose and lipid oxidation were calculated from oxygen consumption and carbon dioxide production data obtained from indirect calorimetry using formulas described previously (33). Nonoxidative glucose disposal, an index of glycogen formation, was calculated by subtracting the rate of glucose oxidation from the rate of TGD. Total-body water was calculated from the mean of the plasma 3-^{3}H-water radioactivity measurements at 90, 105, and 120

min after the intravenous bolus of ^{3}H$_2$O. Plasma tritiated water specific activity was calculated assuming that plasma volume represents 93% of total volume. FFM was calculated by dividing total-body water by 0.73 (34).

The area under the glucose, insulin, C-peptide, and FFA curves during the OGTT were determined using the trapezoidal rule. The mean plasma glucose, insulin, C-peptide, and FFA concentrations during the OGTT were calculated by dividing the area under the curve by the duration of the OGTT (120 min). The insulinogenic index was calculated as the increment in plasma insulin concentration divided by the increment in plasma glucose concentration during the 0- to 30- and 0- to 120-min time periods during the OGTT (20).

Statistical analysis. Statistics were performed with StatView for Windows, version 5.0 (SAS Institute, Cary, NC). Comparison of values before and after insulin infusion were made using the paired Student's t test. Because rosiglitazone increased insulin-stimulated glucose disposal and IRS-1 tyrosine phosphorylation similarly in the diet- and sulfonylurea-treated groups, all 20 diabetic subjects were analyzed collectively. Comparison of variables during the OGTT and during the insulin clamp between before and after rosiglitazone treatment was made using repeated-measures ANOVA with Bonferroni/Dunn post hoc testing. Comparison of variables between control and diabetic subjects were made using ANOVA. Linear regression analysis was used to examine the relationship between measured variables. All data are presented as means $\pm$ SE. A P value <0.05 was considered statistically significant.

RESULTS

Glycemic control and lipids levels. After 16 weeks of rosiglitazone treatment, body weight, BMI, and fat mass increased, whereas plasma HbA$_{1c}$ and FPG, insulin, and FFA concentrations decreased significantly. Rosiglitazone therapy was associated with significant increases in both the fasting plasma HDL and LDL cholesterol concentrations. After rosiglitazone treatment, the mean plasma glucose ($P < 0.0001$) and FFA ($P < 0.0001$) concentrations during the OGTT decreased significantly without change in the mean plasma insulin or C-peptide concentrations. The decrement in mean plasma glucose concentration (65 $\pm$ 11) during the OGTT was significantly greater ($P < 0.01$) than the decrement in FPG concentration (46 $\pm$ 9), indicating that rosiglitazone had a specific effect to reduce postprandial hyperglycemia. Although there was no difference in the plasma insulin excursion during the OGTT performed before and after rosiglitazone treatment, the insulinogenic index (0–120 min) increased significantly
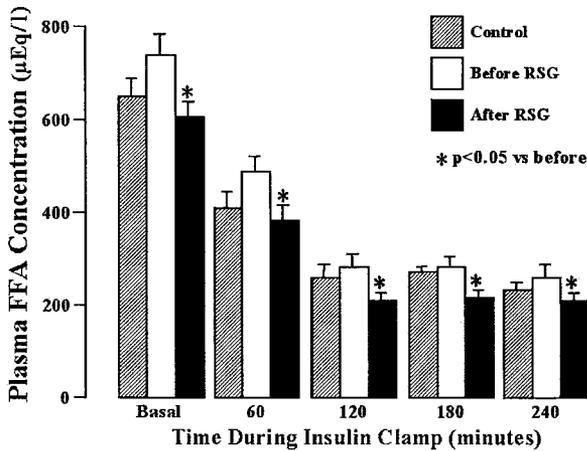
FIG. 2. Time-related change in plasma FFA concentrations during the insulin clamp before and after rosiglitazone (RSG) treatment in type 2 diabetic patients.

($P = 0.04$) after rosiglitazone treatment (Table 1 and Fig. 1).

Euglycemic insulin clamp with muscle biopsy
Plasma glucose, insulin, and FFA concentrations. During the euglycemic insulin clamp studies performed before and after rosiglitazone, the mean steady-state plasma glucose concentrations were 99 ± 1 and 99 ± 1 mg/dl, with coefficients of variation of 3.1 ± 0.5 and $3.0 \pm 0.4\%$, respectively. In all diabetic subjects, the desired glycemic goal (100 mg/dl) was reached within 90 min after the start of insulin infusion. The steady-state plasma insulin concentrations during the euglycemic clamp were similar before and after rosiglitazone treatment (123 ± 9 vs. 114 ± 9 μU/ml, respectively). At all time points during the euglycemic insulin clamp, the suppression of the plasma FFA concentration was significantly greater during the study performed after rosiglitazone treatment (Fig. 2). In control subjects, the steady-state plasma glucose and insulin concentrations during the insulin clamp were 99 ± 1 mg/dl and 103 ± 5 μU/ml, respectively. The suppression of plasma FFAs during the insulin clamp in control subjects was greater than in diabetic subjects before rosiglitazone at all time points.

EGP. After 16 weeks of rosiglitazone treatment the basal rate of EGP decreased significantly from 3.5 ± 0.2 to 3.0 ± 0.1 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1} ($P < 0.01$), and the decrement in basal EGP was correlated ($r = 0.45$, $P < 0.05$) with the decrement in FPG concentration. During the hyperinsulinemic-euglycemic clamp performed before rosiglitazone, EGP was suppressed to 17% (0.6 ± 0.2 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}). After rosiglitazone treatment, the suppression of EGP during the insulin clamp was more complete: 0.2 ± 0.1 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1} ($P < 0.05$ vs. pre-rosiglitazone). In control subjects, the basal EGP was 2.8 ± 0.2 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1} and declined to 0.2 ± 0.1 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1} during the insulin clamp (Fig. 3).

TGD during the insulin clamp. After rosiglitazone treatment, both the TGD (6.9 ± 0.7 to 9.2 ± 0.8 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}, $P < 0.001$) and MCR of glucose (7.0 ± 0.7 to 9.2 ± 0.8 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}, $P < 0.001$) increased by 33 and 31%, respectively, but still remained less than in control subjects (11.1 ± 0.9 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1} and 11.2 ± 0.9 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}, respectively). In 2 of the

20 diabetic subjects, insulin-mediated MCR of glucose did not increase, and in these two subjects, there was no decrease in HbA$_{1c}$. The increase in TGD in diabetic subjects after rosiglitazone treatment was accounted for by an increase in nonoxidative glucose disposal (NOGD) (3.6 ± 0.7 to 5.6 ± 0.7, $P < 0.001$). The glucose oxidation rate did not change significantly (3.3 ± 0.2 to 3.6 ± 0.2 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}, NS). In diabetic subjects treated with rosiglitazone, NOGD still remained less than in control subjects (7.6 ± 0.9 mg $\cdot$ kg FFM^{-1} $\cdot$ min^{-1}). The increase in insulin-mediated TGD after rosiglitazone treatment was strongly correlated with the decline in postprandial glucose excursion (ΔTGD vs. Δmean plasma glucose during the OGTT, $r = -0.74$, $P = 0.0002$).

Insulin receptor signaling. Basal and insulin-stimulated values for insulin receptor and IRS-1 tyrosine phosphorylation for the amount of p85 associated with IRS-1 and for IRS-1–associated PI 3-kinase activity are shown in Figs. 4 and 5. All values are expressed as the percent of the mean insulin-stimulated value in control subjects. Under postabsorptive conditions, insulin receptor tyrosine phosphorylation tended to be higher in diabetic versus control subjects, consistent with previous observations from our laboratory (4). During the insulin clamp performed before rosiglitazone, hyperinsulinemia did not significantly increase insulin receptor tyrosine phosphorylation, but IRS-1 tyrosine phosphorylation and the amount of p85 and PI 3-kinase activity associated with IRS-1 rose slightly ($P < 0.05$). Compared with the nondiabetic control group, the insulin-stimulated increases in insulin receptor and IRS-1 tyrosine phosphorylation and the amount of p85 and PI 3-kinase activity associated with IRS-1 were significantly lower ($P < 0.01$) in diabetic subjects. During the insulin clamp performed after 4 months of rosiglitazone treatment, IRS-1 tyrosine phosphorylation ($P < 0.01$) and the amount of p85 ($P < 0.05$) and PI 3-kinase activity ($P < 0.05$) associated with IRS-1 increased significantly without improvement in insulin-stimulated insulin receptor tyrosine phosphorylation. After rosiglitazone, the incremental response in insulin-stimulated IRS-1 tyrosine phosphorylation and PI 3-kinase activity associated with IRS-1 was similar to the responses in control subjects.

Relationship between TGD and IRS-1 tyrosine phosphorylation and plasma FFA concentrations.
TGD and NOGD during the insulin clamp correlated positively with the insulin-stimulated increment in IRS-1 tyrosine phos-
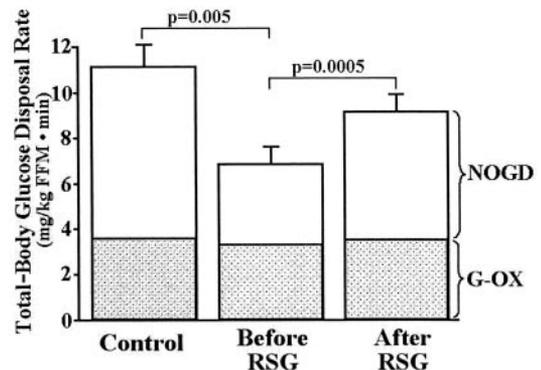

FIG. 3. TGD, glucose oxidation rate (G-OX), and NOGD during the euglycemic insulin clamp performed before and after rosiglitazone (RSG) treatment in type 2 diabetic patients.
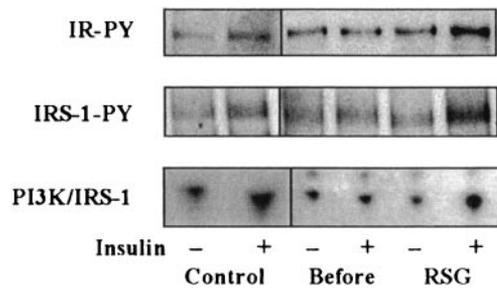
FIG. 4. Representative immunoblots. Effect of rosiglitazone (RSG) on insulin-stimulated insulin receptor (IR) and IRS-1 tyrosine phosphorylation (PY) and the association of PI 3-kinase activity with IRS-1 (PI3-K/IRS-1). There was no change in either IR or IRS-1 protein content.

phorylation ($r = 0.52$, $P = 0.0006$ and $r = 0.49$, $P = 0.001$, respectively) and inversely with the plasma FFA concentration ($r = -0.50$, $P = 0.0006$ and $r = -0.48$, $P = 0.001$, respectively) in control and diabetic subjects before and after rosiglitazone treatment (Fig. 6). TGD and NOGD also correlated with the insulin-stimulated increment in the association of p85 with IRS-1 ($r = 0.38$, $P = 0.01$ and $r = 0.41$, $P = 0.006$, respectively). Neither the fasting plasma FFA concentration nor the plasma FFA concentration during the insulin clamp correlated with the insulin-stimulated increment in IRS-1 tyrosine phosphorylation or the association of p85 with IRS-1.

DISCUSSION

In the present study, 16 weeks of rosiglitazone treatment in type 2 diabetic patients improved glycemic control ($\Delta HbA_{1c} = 1.4\%$) by suppressing basal hepatic glucose production, improving insulin-mediated glucose disposal in muscle (Fig. 3), and enhancing β-cell function (insulinogenic index, 0–120 min) (Table 1). The improvement in β-cell function could represent a direct effect of the TZD on the β-cell (35) or could occur secondary to amelioration of glucose toxicity (2,36) or lipotoxicity (2,37,38), i.e., decreased fasting and/or postprandial FFA levels (Table 1 and Fig. 1). The clinical relevance of these physiological findings are underscored by the following: 1) the decline in

basal EGP was correlated with the reduction in FPG concentration and 2) the improvement in insulin-mediated TGD (which primarily represents muscle [2]) was strongly correlated with the decline in postprandial glucose excursion (ΔTGD vs. Δmean plasma glucose during the OGTT, $r = -0.74$, $P = 0.0002$).

Consistent with the improvement in peripheral tissue (muscle) sensitivity to insulin, 16 weeks of rosiglitazone treatment enhanced insulin-stimulated IRS-1 tyrosine phosphorylation and increased the association of the p85 regulatory subunit of PI 3-kinase, as well as PI 3-kinase activity, with IRS-1 (Figs. 4 and 5). Moreover, insulin-stimulated TGD/NOGD rates correlated well with the insulin-stimulated increments in IRS-1 tyrosine phosphorylation and the association of p85 of PI 3-kinase with IRS-1 before and after rosiglitazone therapy (Fig. 6). The biochemical and molecular mechanism(s) via which rosiglitazone improves muscle sensitivity to insulin have yet to be defined, but some insights are gained from the present results. To initiate their action, TZDs must first bind to PPAR-γ (11–13). PPAR-γ is predominantly expressed in adipose tissue, and its expression in skeletal muscle is low—only ~10% of the expression observed in adipose tissue (14,39). This tissue distribution raises some questions about how rosiglitazone (Fig. 3) and other TZDs (17,19,20) improve insulin-mediated glucose metabolism in skeletal muscle, the tissue responsible for ~70–80% of insulin-stimulated glucose disposal.

Recent studies (22–24,40,41) have underscored the original observations of Randle et al. (42) that elevated circulating FFA levels lead to insulin resistance. This may be of particular importance with respect to the TZDs, because numerous studies have demonstrated that treatment of type 2 diabetic patients with this class of drugs causes a 20–30% reduction in fasting plasma FFA concentration and plasma FFA turnover, which is paralleled by a 20–30% reduction in FPG concentration and HbA_{1c} (17–20,43). The decrease in circulating plasma FFA concentration results both from an inhibition of lipolysis and an increased uptake of FFA in adipose tissue after activation of PPAR-γ
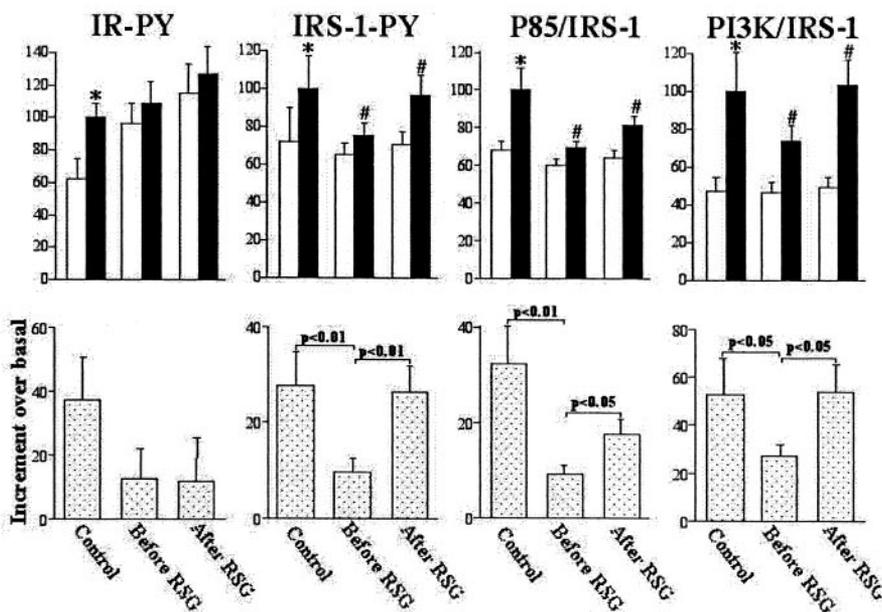

FIG. 5. Effect of rosiglitazone treatment in type 2 diabetic patients on insulin-stimulated insulin receptor tyrosine phosphorylation (IR-PY), IRS-1 tyrosine phosphorylation (IRS-1-PY), amount of p85 associated with IRS-1 (p85/IRS-1), and association of PI 3-kinase activity with IRS-1 (PI3K/IRS-1). Comparative data in nondiabetic control subjects are shown in the first bar. Determinations were made on vastus lateralis muscle biopsies taken during the basal state (□) and after 30 min of insulin infusion (■). Data are expressed as the percent of the mean insulin-stimulated value in healthy control subjects (upper panel). The insulin-stimulated increments in IR-PY, IRS-1-PY, p85/IRS-1, and PI3K/IRS-1 are shown in the lower panel. *$P < 0.05$ vs. basal; #$P < 0.01$ vs. basal.
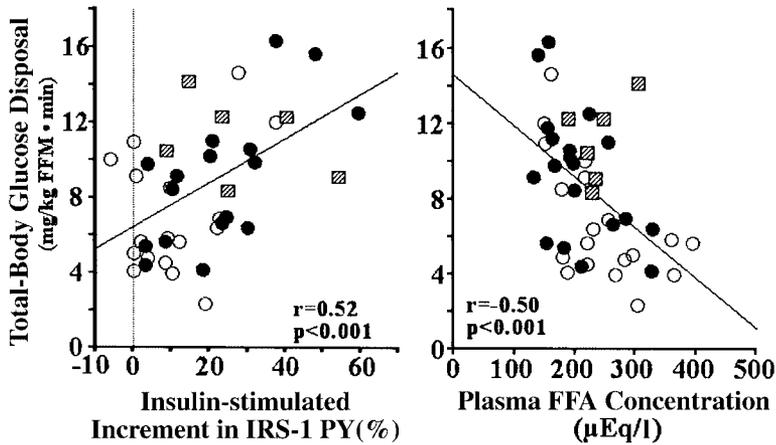
FIG. 6. Relationship between TGD and the insulin-stimulated increment in IRS-1 tyrosine phosphorylation (IRS-1-PY) and the plasma FFA concentration in control subjects (▨) and diabetic subjects before (○) and after (●) rosiglitazone treatment.

(11,20,44). Our results demonstrate that rosiglitazone treatment decreases the fasting and post-OGTT FFA concentrations and improves the suppression of plasma FFA concentration in response to physiological hyperinsulinemia during the euglycemic insulin clamp (Fig. 2). Furthermore, the fasting plasma FFA concentration, as well as the plasma FFA concentration during the OGTT and during the insulin clamp, correlated inversely with peripheral tissue (muscle) insulin sensitivity. However, these FFA concentrations (fasting, post-OGTT, and insulin clamp) were not correlated with insulin-stimulated increments in insulin receptor tyrosine phosphorylation, IRS-1 tyrosine phosphorylation, or the association of p85 or PI 3-kinase activity with IRS-1 after rosiglitazone treatment. These results can be interpreted in several ways. *1*) The improvement in insulin signaling after rosiglitazone treatment is not causally related to a decline in plasma FFA concentration and that rosiglitazone exerts parallel but separate effects to reduce plasma FFA levels and to enhance insulin signaling. It could be argued that the small number of PPAR-γ receptors in muscle are sufficient to activate the insulin signal transduction cascade, whereas the stimulatory effect of rosiglitazone on fat cell PPAR-γ is responsible for the decline in plasma FFA concentration. *2*) Alternatively, it is possible that the rosiglitazone-induced decline in basal and post-OGTT FFA levels is causally related to the improvement in insulin signaling but that the actual cellular mediator responsible for the increased insulin signaling is a metabolic product of FFA metabolism, such as fatty acyl-CoA (45) or diacylglycerol (26) or some intracellular molecule whose formation is stimulated by FFA or fatty acyl-CoA, such as ceramide (46). In support of this hypothesis is the strong correlation between the reductions in basal/post-OGTT/insulin clamp FFA levels and improved muscle insulin sensitivity during the insulin clamp and improved glucose tolerance/insulin sensitivity index during the OGTT. *3*) The improvement in insulin signal transduction could result from the reduction in plasma glucose concentration and amelioration of glucose toxicity (36), or *4*) enhanced insulin signaling could reflect both direct (mediated via PPAR-γ in muscle) and indirect (mediated via the reduction in plasma glucose and FFA concentration/intracellular metabolites of FFA) effects of rosiglitazone. Although several studies have found that mRNA levels of PPAR-γ in skeletal muscle are much less than in adipose tissue (14,39), a recent study demonstrated that PPAR-γ expression is similar in skeletal muscle and adipose tissue (47), supporting a possible direct effect of rosiglitazone on skeletal muscle.

Under basal conditions, insulin receptor tyrosine phosphorylation was increased in type 2 diabetic subjects compared with control subjects, and insulin-stimulated (insulin clamp) tyrosine phosphorylation of the insulin receptor was impaired in type 2 diabetic subjects. A high basal and decreased insulin-stimulated tyrosine phosphorylation of the insulin receptor in skeletal muscle has previously been described by us in insulin-resistant type 2 diabetic subjects (4) and in insulin-resistant nondiabetic humans (48). It is noteworthy that, in the present study, rosiglitazone treatment enhanced downstream insulin receptor signaling (IRS-1 tyrosine phosphorylation and amount of p85 and PI 3-kinase activity associated with IRS-1) without any improvement in insulin-stimulated tyrosine phosphorylation of the insulin receptor in diabetic subjects. These results suggest that rosiglitazone might enhance the amplification of a small signal from the insulin receptor to IRS-1 or might have a direct effect on IRS-1 tyrosine phosphorylation. Further studies will be needed to determine the precise mechanism/site via which rosiglitazone improves insulin signal transduction.

Rosiglitazone reduced the mean day-long plasma glucose concentration by decreasing both the fasting and postprandial glucose levels (Fig. 1). Chronic hyperglycemia has been shown to induce insulin resistance in muscle, a phenomenon referred to as glucose toxicity (36). Conversely, a reduction in plasma glucose concentration by sulfonylurea drugs (49) and phlorizin (50) improves insulin sensitivity in muscle. The deleterious effect of hyperglycemia on muscle insulin sensitivity has been shown to be closely related to a downregulation of GLUT4 transport system (50). We believe that amelioration of glucose toxicity is unlikely to explain the rosiglitazone-enhanced insulin-signaling activity observed in the present study, because the improvement in muscle sensitivity to insulin after the reduction in glucose toxicity occurs in the absence of any effect on the insulin signal transduction system. Kim et al. (29) demonstrated that 16 weeks of troglitazone, but not metformin, therapy in type 2 diabetic patients enhanced insulin-stimulated PI 3-kinase activity in skeletal muscle biopsies, although both troglitazone and metformin improved insulin-stimulated TGD and glycemic control similarly in type 2 diabetic patients. These results

suggest that TZDs enhance the insulin-signaling cascade in muscle and that this effect cannot be explained by removal of glucose toxicity, because improved insulin signaling was not observed in the metformin-treated group. However, this study (29) cannot distinguish between a direct effect of troglitazone on the insulin-signaling cascade versus an indirect effect mediated via a reduction in plasma FFA levels (25,26). Moreover, the infusion rate of insulin ($300 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) used by these investigators (29) produced markedly pharmacological elevations in the plasma insulin concentration, making the physiological meaning of these results difficult to interpret. Our results, which used physiological plasma insulin concentrations and a different TZD (rosiglitazone), are consistent with those of Kim et al. (29) and indicate that, throughout the physiological and pharmacological range of plasma insulin concentrations, TZDs improve insulin-stimulated insulin signal transduction and tissue glucose disposal in vivo.

In the present study, 16 weeks of rosiglitazone treatment was associated with a significant increase in insulin-mediated NOGD, which primarily represents glycogen synthesis (51). Glucose oxidation was not enhanced by rosiglitazone treatment. Our results suggest that rosiglitazone improves NOGD via two distinct mechanisms: enhanced insulin-signaling transduction (IRS-1 tyrosine phosphorylation and the association of p85 and PI 3-kinase activity with IRS-1) and the reduction in circulating FFA levels. Both of these metabolic parameters were significantly correlated with the NOGD rate in the present study. A number of studies have demonstrated that high plasma FFA levels inhibit insulin-stimulated glycogen synthesis, in association with decreased glycogen synthase activity (22,23,28,52). Increased fatty acyl-CoAs also have been shown to directly inhibit glycogen synthase activity in vitro (53).

In summary, 16 weeks of rosiglitazone treatment improved glycemic control ($\Delta HbA_{1c} = 1.4\%$) in type 2 diabetic patients by augmenting peripheral (muscle) and hepatic insulin sensitivity and by enhancing β-cell function. The improvement in peripheral (muscle) insulin sensitivity after rosiglitazone treatment is accounted for entirely by an increase in NOGD (glycogen synthesis). Our results suggest that the improvement in insulin-stimulated muscle glucose disposal after rosiglitazone therapy results from two separate effects of TZDs: 1) enhanced insulin-stimulated insulin signal transduction (increased IRS-1 tyrosine phosphorylation and the association of p85/PI 3-kinase activity with IRS-1) and 2) chronic reduction in circulating plasma FFA levels. Alternatively, it is possible that the reduction in plasma FFA and intracellular FFA metabolites is responsible for both the improvements in insulin signal transduction and insulin-mediated glucose disposal and that significant correlations between the reduction in plasma FFA concentration and insulin-signaling events could not be detected because of individual patient variability and because intracellular fatty acyl-CoAs/other FFA metabolites were not measured.

REFERENCES

1. DeFronzo RA: Lilly Lecture 1987: The triumvirate: β-cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* 37:667–687, 1988
2. DeFronzo RA: Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetic genes. *Diabetes Reviews* 4:177–269, 1997
3. Zierath JR, Krook A, Wallberg-Henriksson H: Insulin action and insulin resistance in human skeletal muscle. *Diabetologia* 43:821–835, 2000
4. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ: Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest* 105:311–320, 2000
5. Saltiel AR, Kahn CR: Insulin signaling and the regulation of glucose and lipid metabolism. *Nature* 414:799–806, 2001
6. Shepherd PR, Kahn BB: Glucose transport and insulin action. *N Engl J Med* 341:248–257, 1999
7. Bonadonna RC, Del Prato S, Bonora E, Saccomani MP, Gulli G, Natali A, Frascerra S, Pecori N, Ferrannini E, Bier D, Cobelli C, DeFronzo RA: Role of glucose transport and glucose phosphorylation in muscle insulin resistance of NIDDM. *Diabetes* 45:915–925, 1996
8. 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
9. Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathsen A, Schneider R, the Pioglitazone 001 Study Group: Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study. *Diabetes Care* 23:1605–1611, 2000
10. Phillips L, Grunberger G, Miller E, Patwardhan R, Rappaport EB, Salzman A, the Rosiglitazone Clinical Trial Study Group: Once- and twice-daily dosing with rosiglitazone improves glycemic control in patients with type 2 diabetes. *Diabetes Care* 24:308–315, 2001
11. Spiegelman BM: PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47:507–514, 1998
12. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkinson WO, Willson TM, Kliewer SA: An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator–activated receptor γ (PPARγ). *J Biol Chem* 270:12953–12956, 1995
13. Berger J, Bailey P, Biswas C, Cullinan CA, Doebber TW, Hayes NS, Saperstein R, Smith RG, Leibowitz MD: Thiazolidinediones produce a conformational change in peroxisomal proliferator–activated receptor-γ: binding and activation correlate with antidiabetic actions in db/db mice. *Endocrinology* 137:4189–4195, 1996
14. Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, Flier JS: Peroxisome proliferator–activated receptor gene expression in human tissue: effects of obesity weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest* 99:2416–2422, 1997
15. Hallakau S, Doare F, Foufelle F, Kergost M, Guerre-Millo, Berthault MF, Dugail I, Mortin J, Auwerx J, Farre P: Pioglitazone induces in vivo adipocyte differentiation in obese Zucker *fa/fa* rats. *Diabetes* 46:1393–1399, 1997
16. Lambe KG, Tugwood JD: A human peroxisome-proliferator–activated receptor γ is activated by inducers of adipogenesis, including thiazolidinedione drugs. *Eur J Biochem* 239:1–7, 1996
17. Maggs DG, Buchanan TA, Burant CF, Cline G, Gumbiner B, Hsueh WA, Inzucchi S, Kelley D, Nolan J, Olefsky JM, Polonsky KS, Silver D, Valiquett TR, Shulman GI: Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 128:176–185, 1998
18. Patel J, Anderson RJ, Rappaport EB: Rosiglitazone monotherapy improves glycaemic control in patients with type 2 diabetes: a twelve-week, randomized, placebo-controlled study. *Diabetes Obes Metab* 1:165–172, 1999
19. Miyazaki Y, Mahankali A, Matsuda M, Mahankali S, Ferrannini E, Cusi K, Mandarino LJ, DeFronzo RA: Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. *Diabetes Care* 24:710–719, 2001
20. Miyazaki Y, Glass L, Triplitt C, Matsuda M, Cusi K, Mandarino LJ, DeFronzo RA: Effect of rosiglitazone on glucose and non-esterified fatty

acid metabolism in type 2 diabetic patients. *Diabetologia* 44:2210–2219, 2001

21. Miyazaki Y, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarino LJ, DeFronzo RA: Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab* 87:2784–2791, 2002

22. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997

23. Thiebaud D, DeFronzo RA, Jacot E, Golay A, Acheson K, Maeder E, Jequier E, Felder JP: Effect of long chain triglyceride infusion on glucose metabolism in man. *Metabolism* 31:1128–1136, 1982

24. Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA: Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* 72:1737–1747, 1983

25. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI: Effects of free fatty acids on glucose transport and IRS-1 associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103:253–259, 1999

26. Itani SI, Ruderman NB, Schmieder F, Boden G: Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IκB-α. *Diabetes* 51:2005–2011, 2002

27. Pendergrass M, Gianluca N, DeFronzo RA: In vivo glucose transport and phosphorylation in skeletal muscle are impaired by elevation of plasma FFA (Abstract). *Diabetes* 47 (Suppl. 1):A65, 1998

28. Kelley DE, Simoneau MM, Mandarino LJ: Interaction between glucose and free fatty acid metabolism in human skeletal muscle. *J Clin Invest* 92:91–98, 1993

29. Kim YB, Ciaraldi TP, Kong A, Kim D, Chu N, Mohideen P, Mudaliar S, Henry RR, Kahn BB: Troglitazone but not metformin restores insulin-stimulated phosphoinositide 3-kinase activity and increases p110β protein levels in skeletal muscle of type 2 diabetic subjects. *Diabetes* 51:443–448, 2002

30. Bonora E, DelPrato S, Bonadonna RC, Gulli G, Solini A, Shank ML, Ghiatas AA, Lancaster JL, Kilcoyne RF, Alyassin AM, DeFronzo RA: Total body fat content and fat topography was associated differently with in vivo glucose metabolism in nonobese and obese nondiabetic women. *Diabetes* 41:1151–1159, 1992

31. Mandarino LJ, Wright K, Verity L, Nichols J, Bell J, Kolterman O, Beck-Nielsen H: Effect of insulin infusion on human skeletal muscle pyruvate dehydrogenase, phosphofructokinase and glycogen synthase. *J Clin Invest* 80:655–663, 1987

32. Steele R: Influence of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 82:420–430, 1959

33. Simonson DC, DeFronzo RA: Indirect calorimetry: methodological and interpretative problems. *Am J Physiol* 258:E399–E412, 1990

34. Hume R, Weyers E: Relationship between total body water and surface area in normal and obese subjects. *J Clin Path* 24:234–238, 1971

35. Dubois M, Pattou F, Kerr-Conte J, Gmyr V, Vandewalle B, Desreumaux P, Auwerx J, Schoonjans K, Lefebvre J: Expression of peroxisome proliferator–activated receptor gamma (PPARgamma) in normal human pancreatic islet cells. *Diabetologia* 43:1165–1169, 2000

36. Rossetti L, Giaccari A, DeFronzo RA: Glucose toxicity. *Diabetes Care* 13:610–630, 1990

37. McGarry JD, Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 1999

38. Shimabukuro M, Zhou YT, Levi M, Unger RH: Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. *J Biol Chem* 273:3547–3550, 1998

39. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J: The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem* 272:18779–18789, 1997

40. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanism of free fatty acid induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994

41. McGarry JD: Banting Lecture 2001: Dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 51:7–18, 2002

42. Randle PJ, Garland PB, Hales CN, Newsholme EA: The glucose fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1:785–789, 1963

43. Suter SL, Nolan JJ, Wallace P, Gumbiner B, Olefsky JM: Metabolic effects of new oral hypoglycemic agent CS-045 in NIDDM subjects. *Diabetes Care* 15:193–203, 1992

44. Way JM, Harrington WW, Brown KK, Gottschalk WK, Sundseth SS, Mansfield TA, Ramachandran RK, Willson TM, Kliewer S: Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor γ activation has coordinate effects on gene expression in multiple insulin-sensitive tissues. *Endocrinology* 142:1269–1277, 2001

45. Prentki M, Corkey BE: Are the β-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 45:273–283, 1996

46. Schmitz-Peiffer C, Criag DL, Biden TJ: Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate. *J Biol Chem* 274:24202–24210, 1999

47. Loviscach M, Rehman N, Carter L, Mudaliar S, Mohadeen P, Ciaraldi TP, Veerkamp JH, Henry RR: Distribution of peroxisome proliferator–activated receptors (PPARs) in human skeletal muscle and adipose tissue: relation to insulin action. *Diabetologia* 43:304–311, 2000

48. Stefan N, Vozarova B, Funahashi T, Matsuzawa Y, Weyer C, Lindsay RS, Youngren JF, Havel PJ, Pratley RE, Bogardus C, Tataranni PA: Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. *Diabetes* 50:1884–1888, 2002

49. Simonson D, Ferrannini E, Bevilaqua S, Smith D, Barett E, Carlson R, DeFronzo RA: Mechanism of improvement in glucose metabolism following chronic glyburide therapy. *Diabetes* 33:838–845, 1984

50. Kahn BB, Shulman GI, DeFronzo RA, Cushman SW, Rossetti L: Normalization of blood glucose in diabetic rats with phlorizin treatment reverses insulin-resistant glucose transport in adipose cells without restoring glucose transport gene expression. *J Clin Invest* 87:561–570, 1991

51. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG: Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 322:223–228, 1990

52. Park JY, Kim CH, Hong SK, Suh KI, Lee KU: Effects of FFA on insulin-stimulated glucose fluxes and muscle glycogen synthase activity in rats. *Am J Physiol* 275:E338–E344, 1998

53. Wititsuwannakul D, Kim K: Mechanism of palmityl coenzyme A inhibition or liver glycogen synthase. *J Biol Chem* 252:7812–7817, 1997