Improved Glycemic Control and Enhanced Insulin Sensitivity in Type 2 Diabetic Subjects Treated With Pioglitazone

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OBJECTIVE — To elucidate the effects of pioglitazone treatment on glucose and lipid metabolism in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 23 diabetic patients (age 30–70 years, BMI < 36 kg/m^2) who were being treated with a stable dose of sulfonylurea were randomly assigned to receive either placebo ($n = 11$) or pioglitazone (45 mg/day) ($n = 12$) for 16 weeks. Before and after 16 weeks of treatment, all subjects received a 75-g oral glucose tolerance test (OGTT); and hepatic and peripheral insulin sensitivity was measured with a two-step euglycemic insulin (40 and 160 mU · min^{-1} · m^{-2}) clamp performed with 3-[^{3}H]glucose and indirect calorimetry. HbA_{1c} was measured monthly throughout the study period.

RESULTS — After 16 weeks of pioglitazone treatment, the fasting plasma glucose (FPG; 184 ± 15 to 135 ± 11 mg/dl, $P < 0.01$), mean plasma glucose during OGTT (293 ± 12 to 225 ± 14 mg/dl, $P < 0.01$), and HbA_{1c} (8.9 ± 0.3 to 7.2 ± 0.5%, $P < 0.01$) decreased significantly without change in fasting or glucose-stimulated insulin/C-peptide concentrations. Fasting plasma free fatty acid (FFA; 647 ± 39 to 478 ± 49 μEq/l, $P < 0.01$) and mean plasma FFA during OGTT (485 ± 30 to 347 ± 33 μEq/l, $P < 0.01$) decreased significantly after pioglitazone treatment. Before and after pioglitazone treatment, basal endogenous glucose production (EGP) and FPG were strongly correlated ($r = 0.67$, $P < 0.01$). EGP during the first insulin clamp step was significantly decreased after pioglitazone treatment ($P < 0.05$), whereas insulin-stimulated total and nonoxidative glucose disposal during the second insulin clamp was increased ($P < 0.01$). The change in FPG was related to the change in basal EGP, EGP during the first insulin clamp step, and total glucose disposal during the second insulin clamp step. The change in mean plasma glucose concentration during the OGTT was strongly related to the change in total body glucose disposal during the second insulin clamp step.

CONCLUSIONS — These results suggest that pioglitazone therapy in type 2 diabetic patients decreases fasting and postprandial plasma glucose levels by improving hepatic and peripheral (muscle) tissue sensitivity to insulin.

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Type 2 diabetes is characterized by defects in both insulin secretion and insulin sensitivity (1,2). The insulin resistance is established early in the natural history of type 2 diabetes (1–3), but with time there is a progressive failure of β-cell function (1,4,5). Based on the pathophysiology of type 2 diabetes, combination therapy with an insulin secretagogue and an insulin sensitizer provides a rational therapeutic approach to reduce blood glucose levels in poorly controlled type 2 diabetic patients (6). Such an approach has been used successfully with sulfonylureas and metformin (7).

Recently, a new class of insulin-sensitizing agents, the thiazolidinediones, was introduced for the treatment of type 2 diabetic patients (8). Troglitazone, the first thiazolidinedione introduced into the U.S. market, has been shown to ameliorate insulin resistance and improve hyperglycemia in patients with type 2 diabetes (9), including sulfonylurea-treated individuals (10). The thiazolidinediones bind to and activate specific nuclear receptors called peroxisome proliferator–activated receptors (PPARs) (8). Stimulation of PPARγ causes the differentiation of preadipocytes into mature fat cells and induces a number of genes involved in lipid synthesis. A close relationship exists between the ability of various thiazolidinediones to activate PPARγ and their hypoglycemic action (8). Pioglitazone, a relatively new member of the thiazolidinedione class, improves hyperglycemia, reduces hyperinsulinemia, and ameliorates hypertriglyceridemia in a variety of insulin-resistant animal models of impaired glucose tolerance (11,12). However, no studies have been published in which the mechanism of the beneficial effects of pioglitazone on glucose and lipid metabolism in diabetic humans has been examined. Moreover, all previous studies that have examined the effect of troglitazone on insulin sensitivity in type 2 diabetic patients used very high insulin infusion rates (120–300 mU · min^{-1} · m^{-2}), which caused a pharmacological elevation in the plasma insulin concentration (9,13,14).

In the present randomized, double-blind, placebo-controlled study, we evaluated the effect of the addition of pioglitazone to sulfonylurea-treated type 2 diabetic patients on glucose tolerance, insulin secretion, hepatic and peripheral insulin sensitivity, and plasma lipid lev-

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Abbreviations: EGP, endogenous glucose production; FFA, free fatty acid; FFM, fat-free mass; FPG, fasting plasma glucose; FPI, fasting plasma insulin; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Changes from baseline in body weight, fat mass, and key substrate/hormone measurements*

	Placebo treatment	Pioglitazone treatment	*P* value
Body weight			
Before	81.4 ± 5.0	84.8 ± 3.6	
After	81.8 ± 5.0	88.4 ± 3.6*	
Change	0.3 ± 0.4	3.6 ± 1.4	0.044
BMI (kg/m^2)			
Before	29.5 ± 1.3	28.7 ± 1.1	
After	29.7 ± 1.3	30.0 ± 1.1*	
Change	0.1 ± 0.2	1.3 ± 0.5	0.037
Fat mass (kg)			
Before	23.7 ± 2.6	26.3 ± 2.6	
After	24.1 ± 2.7	29.8 ± 2.9*	
Change	0.3 ± 0.4	3.5 ± 1.3	0.040
Percentage body fat (%)			
Before	28.8 ± 2.2	30.4 ± 2.0	
After	29.1 ± 2.2	33.1 ± 1.9*	
Change	0.3 ± 0.4	2.7 ± 1.0	0.043
FFM (kg)			
Before	57.7 ± 3.5	58.4 ± 1.6	
After	57.0 ± 3.5	58.6 ± 1.6	
Change	0.0 ± 0.1	0.2 ± 0.2	
FPG (mg/dl)			
Before	159 ± 13	184 ± 15	
After	184 ± 21	135 ± 11†	
Change	25 ± 22	−50 ± 12	0.006
HbA_{1c} (%)			
Before	7.9 ± 0.3	8.9 ± 0.3	
After	8.0 ± 0.4	7.2 ± 0.5†	
Change	0 ± 0.2	−1.7 ± 0.3	<0.001
Fasting insulin (μU/ml)			
Before	21 ± 4	17 ± 3	
After	13 ± 3	15 ± 4	
Change	−3 ± 2	−2 ± 3	
Fasting C-peptide (ng/ml)			
Before	2.6 ± 0.4	2.7 ± 0.3	
After	2.5 ± 0.5	2.5 ± 0.8	
Change	−0.1 ± 0.6	−0.1 ± 0.6	
Total cholesterol (mg/dl)			
Before	172 ± 6	169 ± 5	
After	171 ± 8	162 ± 6	
Change	−1 ± 5	−7 ± 6	
LDL cholesterol (mg/dl)			
Before	111 ± 5	107 ± 4	
After	111 ± 5	105 ± 5	
Change	0 ± 4	−2 ± 6	
HDL cholesterol (mg/dl)			
Before	37 ± 2	35 ± 3	
After	36 ± 2	35 ± 2	
Change	−1 ± 1	1 ± 2	
Triglycerides (mg/dl)			
Before	123 ± 12	138 ± 15	
After	123 ± 15	105 ± 11*	
Change	1 ± 11	−33 ± 11	0.047
FFAs (μEq/l)			
Before	659 ± 47	647 ± 39	
After	672 ± 54	478 ± 49*	
Change	13 ± 52	168 ± 52	0.020

*$P < 0.05$ vs. before treatment; †$P < 0.01$ vs. before treatment.

els. To the best of our knowledge, this represents the first study that has attempted to define the mechanism(s) by which pioglitazone improves glucose metabolism and glycemic control in type 2 diabetic patients, and that has examined the effect of physiologic plasma insulin concentrations on tissue sensitivity to insulin.

RESEARCH DESIGN AND METHODS

Subjects

A total of 23 type 2 diabetic patients were recruited from the outpatient medicine clinic of the Texas Diabetes Institute. All subjects had been taking a stable dose of sulfonylurea for at least 3 months before study. Patients who had previously received insulin, metformin, another thiazolidinedione, or acarbose were excluded. Entry criteria included age 30–70 years, BMI < 36 kg/m^2, stable body weight for at least 3 months before the study, and fasting plasma glucose (FPG) 140–240 mg/dl (Table 1). Patients were in good general health without cardiac, hepatic, renal, or other chronic diseases. Subjects were not consuming any medications known to affect glucose metabolism and none were performing any excessive physical activity. All subjects gave signed, informed consent before participation in the study. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

Study design

The study had a double-blind, placebo-controlled, parallel design. During the 4 weeks before randomization, FPG was measured on four occasions at weekly intervals, and in each subject, the variability was <5%. HbA_{1c}, fasting plasma lipids, and blood pressure were determined during each visit. During this 4-week period, subjects met with the dietitian and were instructed to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein. During the week before randomization, each subject underwent the following: *1*) 75-g oral glucose tolerance test (OGTT); *2*) measurement of lean body mass and fat mass using an intravenous bolus of ^3H_2O; *3*) euglycemic insulin clamp study with tritiated glucose and indirect calorimetry to examine hepatic and peripheral tissue

sensitivity to insulin. The bolus injection of ^3H_2O was performed on the same day as the OGTT, which was performed 4–7 days before the insulin clamp study. All studies were performed in the postabsorptive state at 8:00 A.M. after an overnight 10- to 12-h fast. Subjects omitted the dose of sulfonylurea on the day of study. After completion of these studies, subjects were randomized to receive placebo or pioglitazone, 45 mg/day, with breakfast. Subjects returned to the clinical research center at 8:00 A.M. every 2 weeks for measurement of FPG, body weight, and blood pressure. On each visit, dietary adherence was reinforced. Fasting plasma lipids and HbA_{1c} were measured monthly.

OGTT

Plasma glucose, free fatty acid (FFA), insulin, and C-peptide concentrations were measured at –30, −15, and 0 min. At time 0, subjects ingested 75 g of glucose in 300 ml of orange-flavored water, and plasma glucose, FFA, insulin, and C-peptide concentrations were measured every 15 min for 2 h. At time 0, a 100-μCi intravenous bolus of ^3H_2O was administered and plasma tritiated water radioactivity was determined at 90, 105, and 120 min for calculation of lean body mass and fat mass (15).

Euglycemic insulin clamp

Insulin sensitivity was assessed with a two-step euglycemic insulin clamp (16). At 8:00 A.M., the FPG was measured and a primed (25 μCi × FPG/100) continuous (0.25 μCi/min) infusion of 3-[^{3}H]glucose was started at time –180 min via a catheter placed into an antecubital vein. A second catheter was placed retrogradely into a vein on the dorsum of the hand, which was then placed in a heated box (60°C). Baseline arterialized venous blood samples for determination of plasma 3-[^{3}H]glucose radioactivity and glucose and insulin concentrations were drawn at −30, −20, −10, −5, and 0 min. At time 0, a prime-continuous infusion of human regular insulin (Novolin; Novo Nordisk Pharmaceuticals, Princeton, NJ) was started at 40 mU · min^{-1} · m^{-2} body surface area and continued for 90 min (16). At time 90 min, the insulin space was reprimed and the insulin infusion rate was increased to 160 mU · min^{-1} · m^{-2} for another 90 min. After initiation of the insulin infusion, plasma glucose concentration was allowed to decrease to 90–100 mg/dl, at which level it was maintained with a variable infusion of 20% dextrose.

Throughout the insulin clamp, blood samples for determination of plasma glucose concentration were drawn every 5 min. Blood samples for plasma insulin and 3-[^{3}H]glucose specific activity were collected every 10–15 min. Continuous indirect calorimetry, using a ventilated hood system (Deltatrac II; SensorMedics, Yorba Linda, CA), was performed during the last 40 min of the basal period and during the last 30 min of each insulin infusion step. Urine samples for determination of urea nitrogen excretion were obtained during the 3 h before and after the start of the insulin clamp.

Assays

Plasma glucose concentration was measured 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) were measured by radioimmunoassay. HbA_{1c} was measured by affinity chromatography (Biochemical Methodology, Drower 4,350; Isolab, Akron, OH). Plasma FFA concentration was measured by enzymatic calorimetric quantification (Wako Chemicals, Neuss, Germany). Plasma total cholesterol and triglyceride levels were measured enzymatically (Boehringer-Mannheim, Indianapolis, IN) on an Hitachi 704 autoanalyzer. HDL cholesterol was measured enzymatically on a Hitachi 704 autoanalyzer after precipitation of chylomicrons and VLDL and LDL cholesterol by phosphotungstic acid. LDL cholesterol was calculated using the Friedwald equation. Tritiated glucose specific activity was determined on barium/zinc deproteinized plasma samples.

Calculations

Under steady-state postabsorptive conditions, the rate of endogenous glucose appearance (R_a) is calculated as the 3-[^{3}H]glucose infusion rate (disintegrations per minute per minute) divided by the steady-state plasma 3-[^{3}H]glucose specific activity (disintegrations per minute per milligram). During the insulin clamp, R_a was calculated from Steele's equation (17), using a distribution volume of 250 ml/kg. Endogenous glucose production (EGP) was calculated as: EGP = R_a − the exogenous glucose infusion rate. Total glucose disposal equals the sum of EGP plus the glucose infusion rate. Rates of glucose and lipid oxidation were calculated on the basis of oxygen consumption and carbon dioxide production data obtained from indirect calorimetry. Nonoxidative glucose disposal, an index of glycogen formation, was calculated by subtracting the rate of glucose oxidation from the rate of total body glucose disposal.

Total body water was calculated from the mean plasma [^{3}H]-water radioactivity at 90, 105, and 120 min after the bolus of $^3H_20$. Plasma tritiated water specific activity was calculated assuming that plasma water represents 93% of total plasma volume. Fat-free mass (FFM) equals total body water divided by 0.73 (18).

The area under the glucose, insulin, C-peptide, and FFA curves during the OGTT was 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 120 min.

Statistical analysis

Statistical calculations were performed with StatView for Windows, version 5.0 (SAS Institute, Cary, NC). Values before and after treatment within each group (intragroup) were analyzed using paired Student's *t* test. Comparison between groups (intergroup) was performed using analysis of variance with Bonferroni/Dunn post hoc testing when appropriate. Comparisons over time were made by using repeated-measures analysis of variance. Pearson correlations between continuous variables were used as a measure of association, and χ^2 test was used for comparing proportions between the groups. Data are presented as mean ± SEM. A P value <0.05 was considered statistically significant.

RESULTS

Patient characteristics, HbA_{1c}, and FPG

Demographic characteristics are summarized in Table 1. The placebo- and pioglitazone-treated groups were similar in age (55 ± 4 vs. 54 ± 3 years), race (three Caucasian, seven Mexican-American, one African-American versus five white, six Mexican-American, and one African-

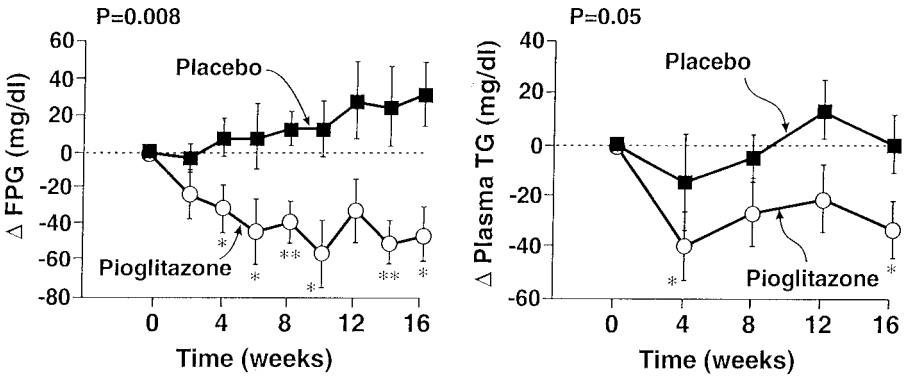

Figure 1—*Time course of the change in FPG and triglyceride (TG) concentrations from baseline during the 4-month study period in pioglitazone and placebo groups.* P *values indicate significant differences in the change of FPG or TG concentrations over the 4-month study period between the two groups.* *P < *0.05 and* **P < *0.01 vs. baseline.*

American), duration of diabetes (4.7 ± 1.4 vs. 5.8 ± 1.4 years), BMI, FPG, HbA_{1c}, fasting insulin and C-peptide concentrations, plasma lipid levels, fat mass, and FFA (Table 1). There were more men than women in the pioglitazone group (11 men, 1 woman) versus the placebo group (6 men, 5 women). However, it is known that women respond better than men to thiazolidinediones because of their greater percentage of body fat (19).

After 16 weeks of treatment, significant increases in body weight, BMI, percentage of body fat, and fat mass were observed in the pioglitazone group (Table 1). Body weight, BMI, and percentage of body fat did not change in the placebo group (Table 1). The changes in body weight, BMI, and fat mass in the pioglitazone group were significantly ($P < 0.05$) greater than in the placebo group. Plasma HbA_{1c} ($\Delta = -1.7 \pm 0.3\%$, $P < 0.01$) and FPG ($\Delta = -50 \pm 12$ mg/dl, $P < 0.01$) decreased significantly in the pioglitazone group and remained unchanged or increased slightly in the placebo group (Table 1). Fasting plasma insulin and C-peptide levels did not change significantly in either group. Fasting plasma triglyceride ($\Delta = -33 \pm 11$ mg/dl, $P < 0.05$) and FFA ($\Delta = -168 \pm 32$ μEq/l, $P < 0.01$) concentrations decreased significantly in the pioglitazone group and remained unchanged in the placebo group. The time-related changes from baseline in FPG and plasma triglyceride concentrations over the 16-week study period are shown in Fig. 1. Within 2 weeks after the start of pioglitazone treatment, the FPG began to decrease and the decrement from baseline achieved statistical significance by week 4. The maximum decrement in FPG was reached after 6 weeks of pioglitazone treatment. In the placebo group, the FPG increased slightly throughout the 16-week study period. Compared with the placebo group, the change in FPG over the 16-week treatment period was significantly greater ($P < 0.01$). The plasma triglyceride concentration decreased significantly by week 4 in the pioglitazone group and remained decreased throughout the 16-week study period ($P < 0.05$). The decrement in plasma triglyceride concentration was greater in the pioglitazone group versus the placebo group ($P = 0.05$).

OGTT

Before treatment, the plasma glucose, insulin, C-peptide, and FFA concentrations during the OGTT were similar in the placebo and pioglitazone groups (Fig. 2). After pioglitazone treatment, the mean plasma glucose (293 ± 12 to 225 ± 14 mg/dl, $P < 0.01$) and FFA (483 ± 30 to 347 ± 33 μEq/l, $P < 0.01$) concentrations during the OGTT decreased significantly from baseline and the decrements were significantly different from the placebo group ($P = 0.001$) (Fig. 2). The incremental area under the plasma glucose concentration curve was reduced by pioglitazone (10,807 ± 761 vs. 13,028 ± 597 mg/dl, $P = 0.01$) and did not change with placebo (11,413 ± 1,360 vs. 11,485 ± 902 mg/dl, NS). There was no significant change in the plasma insulin and C-peptide concentrations during the OGTT after pioglitazone or placebo treatment (Fig. 2). The fasting plasma FFA concentration ($r = 0.325$, $P = 0.03$) and the mean plasma FFA during the OGTT ($r = 0.481$, $P < 0.001$) were correlated with the mean plasma glucose concentration during the OGTT. The fasting plasma FFA concentration was correlated with the FPG ($r = 0.282$, $P = 0.05$).

Euglycemic insulin clamp

Before treatment, the plasma glucose concentrations during the first (103 ± 7 vs. 98 ± 5 mg/dl) and second (90 ± 1 vs. 91 ± 1 mg/dl) insulin clamp steps were similar in the pioglitazone and placebo groups, respectively. The plasma insulin concentrations during the first (69 ± 8 and 77 ± 8 μU/ml) and second (341 ± 28 and 379 ± 29 μU/ml) insulin clamp steps were similar in the pioglitazone and placebo groups. During the first and second insulin clamp steps performed after pioglitazone and placebo treatment, the plasma glucose and insulin concentrations were similar to those in the baseline insulin clamp study.

Before treatment, the basal rate of EGP was similar in the pioglitazone (2.8 ± 0.1 mg/kg FFM per min) and placebo (2.5 ± 0.1 mg/kg FFM per min) groups and remained unchanged after 16 weeks of pioglitazone (2.8 ± 0.1 mg/kg FFM per min) and placebo (2.6 ± 0.1 mg/kg FFM per min) treatment. Before treatment, the FPG concentration was strongly correlated with basal EGP in all subjects ($r = 0.73$, $P < 0.001$), and this correlation persisted after treatment. Because a 3-h period is required for tritiated glucose equilibration before the start of the insulin clamp, basal EGP was measured at ~11:30 A.M., whereas the FPG concentration during the OGTT and bimonthly follow-up visits was measured at 8:00 A.M. During the insulin clamp study performed before the start of therapy, the plasma glucose concentration decreased to 153 ± 10 mg/dl (~11:30 A.M.) in the pioglitazone group and to 156 ± 9 mg/dl (~11:30 A.M.) in the placebo group. During the insulin clamp performed after 4 months, the plasma glucose concentration in the pioglitazone-treated and placebo-treated groups decreased to 130 ± 11 mg/dl (~11:30 A.M.) and to 156 ± 16 mg/dl (~11:30 A.M.), respectively (both $P < 0.01$). The time-related decreases in FPG concentration and EGP with prolongation of the fasting period is related to liver glycogen depletion and decreased glycogenolysis (20).

During the first insulin clamp step,

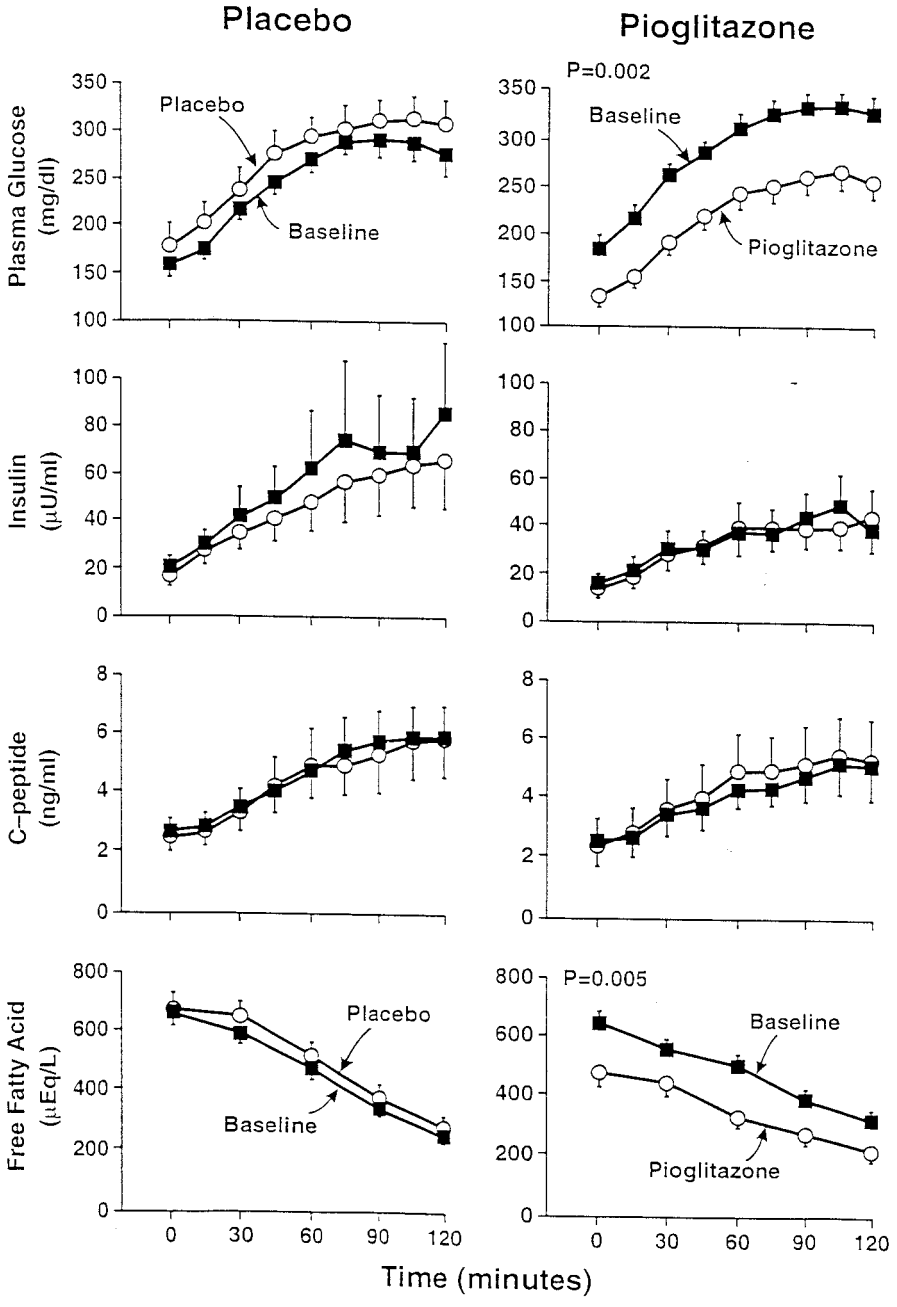

Figure 2—*Plasma glucose, insulin, C-peptide, and FFA concentrations during the 75-g OGTT before and after treatment with placebo or pioglitazone.* P *values indicate significant differences between before and after treatment.*

suppression of EGP after administration of pioglitazone (1.1 ± 0.2 to 0.6 ± 0.2 mg/dl, $P < 0.05$) was greater than with placebo (1.0 ± 0.1 to 1.4 ± 0.2 mg/dl, $P < 0.01$). During the second insulin clamp step, EGP was nearly completely suppressed in both the pioglitazone group (0.4 ± 0.1 mg/dl) and the placebo group (0.3 ± 0.1 mg/dl).

In the basal state, total body glucose disposal, glucose oxidation, and nonoxidative glucose disposal were similar in pioglitazone and placebo groups before and after treatment (Fig. 3). During the initial insulin clamp in the pioglitazone group, insulin-stimulated rates of total body glucose disposal, glucose oxidation, and nonoxidative glucose disposal were similar to those in the placebo group. Four months of placebo treatment had no effect on insulin-mediated total body, oxidative, or nonoxidative glucose disposal (Fig. 3). After 4 months of pioglitazone treatment, there was no significant change in total body glucose disposal (4.2 ± 0.3 vs. 3.9 ± 0.4 mg/kg FFM per min) during the first insulin clamp step and a 38% increase (10.5 ± 1.0 vs. 7.6 ± 1.0, $P < 0.01$ vs. baseline insulin clamp and $P < 0.01$ vs. the placebo group) during the second insulin clamp step (Fig. 3). The improvement in insulin sensitivity was due almost entirely to an improvement in nonoxidative glucose disposal ($P < 0.01$ vs. baseline insulin clamp and $P < 0.01$ vs. placebo) (Fig. 3).

An independent composite measure of whole-body insulin sensitivity can be derived from the OGTT as follows (21):

$$\frac{10{,}000}{\sqrt{(\text{FPG} \times \text{FPI}) \times (\overline{\text{PG}} \times \overline{\text{PI}})}}$$

where FPI represents the fasting plasma insulin concentration and $\overline{\text{PG}}$ and $\overline{\text{PI}}$ represent the mean plasma glucose and plasma insulin concentrations during the OGTT, respectively. This index provides a composite measure of the combined effects of hyperinsulinemia and hyperglycemia on the muscle and liver, and it has been shown to be highly correlated with insulin sensitivity measured with the euglycemic insulin clamp technique (21). In the placebo group, there was no change in the OGTT-derived composite index of insulin sensitivity (2.2 ± 0.4 vs. 2.1 ± 0.3 mg/dl, respectively; NS). In the pioglitazone group, the OGTT-derived composite index of insulin sensitivity rose by 48% from 2.7 ± 0.4 to 4.0 ± 0.5 mg/dl ($P < 0.01$) after 4 months.

Basal and insulin-mediated suppression (first and second insulin clamp steps) of lipid oxidation was similar in placebo (0.9 to 0.7 to 0.3 mg/kg FFM per min) and pioglitazone (1.0 to 0.7 to 0.4 mg/kg FFM per min) groups before treatment and remained unchanged after 16 weeks of treatment.

Blood pressure

Before treatment, there were no differences in systolic blood pressure (130 ± 3 vs. 127 ± 5 mmHg) or diastolic blood pressure (73 ± 4 vs. 77 ± 2 mmHg) between the placebo and pioglitazone groups. During the 16-week study period, there were no significant changes in blood pressure in either group.

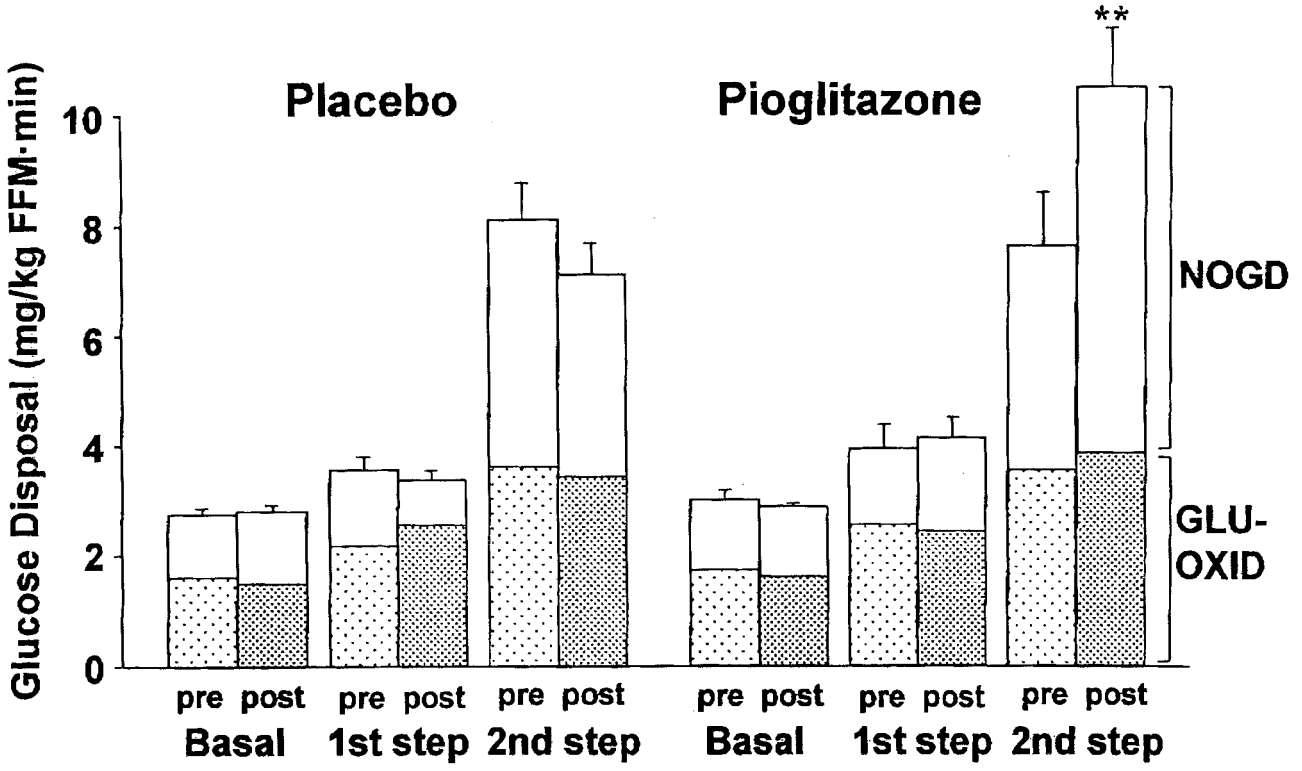

Figure 3—*Rates of total body glucose disposal, glucose oxidation (GLU-OXID), and nonoxidative glucose disposal (NOGD) during the basal state and during the first (40 mU · min^{-1} · m^{-2}) and second (160 mU · min^{-1} · m^{-2}) insulin clamp steps in the placebo (left) and pioglitazone (right) groups before and after 4 months of treatment.*

Regression analyses

Table 2 shows the results of simple Pearson correlation coefficients between the changes in FPG and mean plasma glucose concentration during the OGTT and the following variables: change in body weight and fat mass, indexes of peripheral and hepatic insulin sensitivity during the euglycemic insulin clamp, and plasma FFA levels in the fasting state and during the OGTT. The change in FPG was weakly and inversely correlated with the changes in body weight ($P = 0.07$) and fat mass ($P = 0.07$). The change in FPG also was inversely correlated with insulin-stimulated glucose disposal (second insulin clamp step) and positively correlated with the change in basal endogenous (primarily hepatic) glucose production and suppression of EGP by insulin (first insulin clamp step). The change in mean plasma glucose concentration during the OGTT was inversely correlated with the change in body weight, the change in fat mass, and insulin-stimulated glucose disposal (second insulin clamp step) and positively correlated with the change in EGP (first insulin clamp step). The changes in FPG and mean plasma glucose concentration during the OGTT were correlated with the change in mean plasma FFA concentration during the OGTT. Using stepwise multivariate analysis, the combined changes in basal EGP and in total body glucose disposal during the second insulin clamp step were the best predictors of change in the FPG level ($R^2 = 0.56, P = 0.0003$). Addition of all other variables did not significantly improve the correlation ($R^2 = 0.69$). The change in total body glucose disposal during the second insulin clamp step was the most important predictor of the change in mean plasma glucose concentration during the OGTT ($R^2 = 0.65, P < 0.0001$).

CONCLUSIONS — In the present study, we examined the mechanisms by which pioglitazone reduces the FPG concentration and postprandial glucose levels in type 2 diabetic patients who were being treated with a stable dose of sulfonylurea. To our knowledge, this study represents the first report that has measured the effect of any thiazolidinedione on insulin sensitivity in type 2 diabetic subjects in response to a physiologic increment in plasma insulin concentration. Three previous studies (9,13,14) have examined the effect of troglitazone on insulin sensitivity in type 2 diabetic individuals, and in each of these studies, the investigators used a pharmacological insulin infusion rate (120–300 mU · min^{-1} · m^{-2}) that produced very unphysiological plasma insulin concentrations. Moreover, because EGP is completely suppressed by these pharmacological levels of hyperinsulinemia, it is not possible to evaluate the effect of troglitazone on hepatic insulin sensitivity.

Our results demonstrate that a 16-week course of pioglitazone (45 mg/day) therapy reduces both the FPG concentration and the postprandial glucose excursion during the OGTT, leading to a 1.7% decrease in HbA_{1c}. It is noteworthy that the decrement in the area under the plasma glucose concentration curve during the OGTT after administration of pioglitazone was decreased by 17% ($P = 0.01$ vs. baseline; $P < 0.01$ vs. placebo). Thus, pioglitazone, unlike metformin (7) and sulfonylureas (22), significantly improved the postprandial glucose excursion. Consistent with this observation, the decrement in mean plasma glucose con-

Table 2—*Summary of Pearson correlation coefficients for the changes in FPG concentration and the change in mean plasma glucose concentration during the OGTT with selected variables*

Parameter	Δ FPG	Δ Mean PG during OGTT
Δ Body weight	−0.377‡	−0.423*
Δ Fat mass	−0.377‡	−0.416*
Δ Basal EGP	0.515*	0.140
Δ First EGP	0.545†	0.474*
Δ First TGD	−0.100	−0.160
Δ Second TGD	−0.541†	−0.806†
Δ Fasting plasma FFA	0.180	0.146
Δ Mean FFA during OGTT	0.514*	0.447*
Δ Triglycerides	0.488*	0.316

FPG measured in mg/dl; plasma glucose (PG) measured in mg/dl; FFA measured in μEq/l; EGP measured in mg/kg FFM per min; total glucose disposal (TGP) measured in mg/kg FFM per min. *$P < 0.05$; †$P < 0.01$; ‡$P = 0.07$. Δ, difference between before and after treatment.

centration during the OGTT (68 ± 14 mg/dl) was greater ($P < 0.01$) than the decrement in FPG concentration (50 ± 12 mg/dl) after pioglitazone treatment.

The improvement in postprandial hyperglycemia with pioglitazone could result from *1*) increased insulin secretion; *2*) enhanced tissue sensitivity to insulin; *3*) an improvement in the combined effects of hyperglycemia plus hyperinsulinemia to promote glucose metabolism.

Plasma insulin and C-peptide levels in the fasting state and during the OGTT were unchanged by pioglitazone therapy. The effect of thiazolidinedione treatment on insulin secretion in type 2 diabetic patients is controversial (9,13,14,19,23,24). Some studies have reported decreased plasma insulin and/or C-peptide levels (9,13,19,23), whereas others have failed to observe any change in plasma insulin/C-peptide concentrations (14,24) after chronic administration of thiazolidinedione in type 2 diabetic subjects. We believe that the inconsistent effect of thiazolidinediones on plasma insulin/C-peptide concentrations is explained by two opposing effects: *1*) a decrease in fasting and postprandial glucose concentrations, which reduce glucose toxicity and enhance β-cell function (1); and *2*) an improvement in insulin sensitivity, which leads to a reduction in insulin secretion (1,25). Based on these considerations, it is not surprising that variable results concerning the effect of thiazolidinediones on plasma insulin and C-peptide levels have been reported. Because we failed to observe any change in plasma insulin or C-peptide concentrations after pioglitazone therapy, it is difficult to ascribe the improvement in glucose homeostasis to increased insulin secretion. However, this should not be construed to mean that pioglitazone has no effect on β-cell function. Improved glucose tolerance without change in the plasma insulin or C-peptide concentrations suggests enhanced β-cell function. This is substantiated by the increased insulinogenic index (ΔI/ΔG) at 30 min (0.16 ± 0.04 to 0.28 ± 0.07 mg/dl, $P = 0.04$) and from 0–120 min (0.18 ± 0.06 to 0.26 ± 0.07 mg/dl, $P = 0.06$).

During the first insulin clamp step, which resulted in a physiologic increment in plasma insulin concentration, we failed to observe any improvement in insulin-mediated glucose disposal in pioglitazone-treated subjects. This finding was surprising, and two comments are pertinent. First, the diabetic patients who participated in this study were very insulin-resistant and demonstrated little increase in glucose disposal during the first insulin clamp step performed before the initiation of pioglitazone therapy (Fig. 3). This may have obscured our ability to observe an enhancement in insulin sensitivity after pioglitazone treatment during the lower-dose insulin clamp step. Second, we reviewed the published literature to examine whether other investigators had demonstrated improved insulin sensitivity to a physiologic increment in plasma insulin concentration in type 2 diabetic subjects who were treated with a thiazolidinedione. Unfortunately, in only three thiazolidinedione (troglitazone) studies (9,13,14) in which insulin clamps were performed, the insulin infusion rates (120–300 mU · min^{-1} · m^{-2}) were in the pharmacological range, and the results of these pharmacological insulin clamp studies were similar to our findings during the second insulin clamp step (Fig. 3). Thus, from the present results, it cannot be discerned whether the failure of pioglitazone to enhance insulin sensitivity during the first insulin clamp step is the result of the severe insulin resistance in our patient population or because thiazolidinediones do not exert a major effect to improve peripheral tissue insulin sensitivity in response to physiologic hyperinsulinemia. In either case, it is clear that enhanced peripheral tissue (muscle) sensitivity to insulin cannot explain the significant improvement in the OGTT, because the plasma insulin concentrations during the OGTT (Fig. 2) were similar to those during the first insulin clamp step. The major differences between the OGTT and euglycemic insulin are the presence (OGTT) or absence (insulin clamp) of hyperglycemia and the route of glucose administration. This suggests that pioglitazone *1*) enhanced glucose-mediated glucose uptake or the combined effects of glucose plus insulin to augment glucose disposal (26), the possibility of which is supported by the 48% increase in the whole-body composite insulin sensitivity index calculated during the OGTT; *2*) augmented splanchnic glucose uptake after glucose injection (27), as suggested by the results of Kawamori et al. (28); *3*) more effectively decreased EGP during the OGTT. This latter possibility is consistent with the improvement in insulin-mediated suppression of EGP during the low-dose insulin clamp performed after pioglitazone treatment.

During the second insulin clamp step, pioglitazone significantly enhanced peripheral tissue insulin sensitivity, due to increased nonoxidative glucose disposal, which primarily reflects muscle glycogen synthesis (Fig. 3). The observation that pioglitazone (Fig. 3) and troglitazone (9,13,14) only have been shown to augment peripheral tissue sensitivity at pharmacological plasma insulin concentrations in type 2 diabetic subjects should not be underemphasized. In Pima Indians, maximally insulin-stimulated glucose uptake by muscle in vivo has been shown to predict the development of type 2 diabetes (29). Moreover, the 38% improvement in insulin sensitivity during the second insulin clamp step was closely correlated with the decreased plasma glucose excursion during the OGTT ($r = -0.81$, $P < 0.01$).

Before the initiation of therapy, EGP and the FPG concentration were positively correlated in the placebo-treated and pioglitazone-treated groups ($r = 0.73$, $P < 0.001$), and these correlations persisted after 16 weeks of treatment. This is not surprising, because the rate of EGP is the primary determinant of the FPG (1,30). It was, however, quite sur-

prising to not observe a decrease in EGP after pioglitazone therapy, especially because other thiazolidinediones (i.e., troglitazone) have been shown to reduce glucose production in isolated hepatocytes (31) and in type 2 diabetic subjects (9,13). We believe that our failure to document a decrease in EGP after pioglitazone therapy is more a reflection of the study design than a lack of effect of the thiazolidinedione on the liver. Diabetic subjects in the present study had modest fasting hyperglycemia and, during the 3-h period required for tritiated glucose equilibration, the FPG concentration had decreased to 153 ± 10 and 130 ± 11 mg/dl, respectively, before and after 16 weeks of pioglitazone therapy. We have shown that hepatic glucose production does not increase until the FPG exceeds 140 mg/dl (30), and Jeng et al. (32) have reported an even higher threshold: FPG ~160 mg/dl. Because the FPG had decreased to 153 mg/dl before pioglitazone and 130 mg/dl after pioglitazone, one would have expected the EGP to be within the normal range at the time (~11:30 A.M.) that the measurement of EGP was performed, and this would have obscured any effect of pioglitazone to suppress the basal rate of hepatic glucose release.

Even though we were unable to demonstrate a reduction in EGP after pioglitazone treatment, we believe that our results provide strong evidence that pioglitazone did improve hepatic sensitivity to insulin. In the postabsorptive state, the product of the fasting plasma insulin (FPI) concentration and the EGP provides a direct measure of hepatic insulin resistance and correlates closely with the product of FPI × FPG (21). The inverse of (FPI × FPG), therefore, provides an index of hepatic sensitivity to insulin (21). After 16 weeks of pioglitazone therapy, the hepatic insulin sensitivity index increased by 60% from 0.20 ± 0.04 to 0.32 ± 0.05 $(mg/dl)^{-1} \times (\mu U/ml)^{-1}$ ($P < 0.001$). No improvement in the hepatic insulin sensitivity index (0.17 ± 0.03 to 0.18 ± 0.04) was observed in the placebo-treated group. The results of the euglycemic insulin clamp study provide further support for an insulin-sensitizing effect of pioglitazone on the liver. Thus, during the first insulin clamp step, pioglitazone enhanced the suppression of EGP by insulin by 45% (0.6 vs. 1.1 mg/kg FFM per minute, $P < 0.01$).

Pioglitazone treatment decreased the fasting plasma FFA concentration and augmented the suppression of plasma FFA during the OGTT (Fig. 2); the latter was correlated with the decrease in plasma glucose concentration during the OGTT ($r = 0.45$, $P < 0.05$). Because elevated plasma FFA levels stimulate gluconeogenesis (33) and impair the suppression of hepatic glucose production in vivo (34), this could explain, in part, the improvement in oral glucose tolerance after pioglitazone treatment. Elevated plasma FFA levels also have been shown to decrease splanchnic (hepatic) glucose uptake in type 2 diabetic individuals (35). It is of interest to speculate that the decrease in plasma FFA concentration is associated with mobilization of lipid from muscle, leading to enhanced muscle sensitivity to insulin (36). The decrease in plasma triglyceride concentration after pioglitazone treatment has been reported with other thiazolidinediones (9,13,23) and is most likely multifactorial, resulting from decreased substrate (reduced plasma FFA and glucose concentrations) delivery to the liver and a direct effect on hepatic synthesis of VLDL (37). The failure to observe any increase in LDL cholesterol (Table 1) is consistent with other clinical trials with pioglitazone (38).

In type 2 diabetic subjects, weight gain is associated with worsening of insulin resistance and a deterioration in glycemic control. In the present study, 16 weeks of pioglitazone treatment was associated with a weight gain of 3.6 kg, and this was entirely accounted for by an increase in fat mass of 3.5 kg. Edema was not observed in any of the subjects. Despite the weight gain, oral glucose tolerance, hepatic insulin sensitivity (first insulin clamp step), and peripheral tissue insulin sensitivity (second insulin clamp step) improved significantly. Improved glycemic control (19,24), despite weight gain, has been reported with other thiazolidinediones, including rosiglitazone and troglitazone. Thiazolidinediones bind to a specific class of receptors (the PPARs) (8). Binding of thiazolidinediones to the PPARγ receptor causes preadipocytes to differentiate into mature small adipocytes and induces a number of genes involved in lipogenesis, explaining the increase in body weight (39,40). Although PPARγ receptors are present in visceral adipose tissue in humans, they do not seem to be activated by thiazolidinediones (41). This may explain the recent finding that troglitazone-induced weight gain is associated with an increase in subcutaneous fat tissue and a decrease in visceral abdominal fat content (42). Increased visceral fat is associated with insulin resistance (43). If the pioglitazone-associated weight gain in the present study is associated with increased subcutaneous fat and a simultaneous decrease in visceral abdominal fat, as reported by Kelly et al. (42), this could contribute to the improvement in insulin sensitivity and glucose tolerance. The long-term effects of the increased body weight on metabolic and cardiovascular outcomes remain to be determined. It is important, therefore, to emphasize diet therapy in diabetic patients being treated with thiazolidinediones.

In summary, the present results demonstrate that a 16-week course of pioglitazone therapy decreases the fasting and postprandial glucose levels, reduces the HbA_{1c}, improves hepatic and peripheral tissue sensitivity to insulin, and lowers plasma FFA and triglyceride concentrations despite significant weight gain. These observations contribute to our understanding of the mechanism of action of the thiazolidinediones.

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