

# Effect of Pioglitazone on Abdominal Fat Distribution and Insulin Sensitivity in Type 2 Diabetic Patients

YOSHINORI MIYAZAKI, ARCHANA MAHANKALI, MASAFUMI MATSUDA, SRIKANTH MAHANKALI, JEAN HARDIES, KENNETH CUSI, LAWRENCE J. MANDARINO, AND RALPH A. DEFONZO

University of Texas Health Science Center and Texas Diabetes Institute, San Antonio, Texas 78229

We examined the effect of pioglitazone on abdominal fat distribution to elucidate the mechanisms via which pioglitazone improves insulin resistance in patients with type 2 diabetes mellitus. Thirteen type 2 diabetic patients (nine men and four women; age,  $52 \pm 3$  yr; body mass index,  $29.0 \pm 1.1$  kg/m<sup>2</sup>), who were being treated with a stable dose of sulfonylurea ( $n = 7$ ) or with diet alone ( $n = 6$ ), received pioglitazone (45 mg/d) for 16 wk. Before and after pioglitazone treatment, subjects underwent a 75-g oral glucose tolerance test (OGTT) and two-step euglycemic insulin clamp (insulin infusion rates, 40 and 160 mU/m<sup>2</sup>·min) with [<sup>3</sup>H]glucose. Abdominal fat distribution was evaluated using magnetic resonance imaging at L4–5. After 16 wk of pioglitazone treatment, fasting plasma glucose ( $179 \pm 10$  to  $140 \pm 10$  mg/dl;  $P < 0.01$ ), mean plasma glucose during OGTT ( $295 \pm 13$  to  $233 \pm 14$  mg/dl;  $P < 0.01$ ), and hemoglobin A<sub>1c</sub> ( $8.6 \pm 0.4\%$  to  $7.2 \pm 0.5\%$ ;  $P < 0.01$ ) decreased without a change in fasting or post-OGTT insulin levels. Fasting plasma FFA ( $674 \pm 38$  to  $569 \pm 31$   $\mu$ Eq/liter;  $P < 0.05$ ) and mean plasma FFA ( $539 \pm 20$  to  $396 \pm 29$   $\mu$ Eq/liter;  $P < 0.01$ ) during OGTT decreased after pioglitazone. In the postabsorptive state, hepatic insulin resistance [basal endogenous glucose production (EGP)  $\times$  basal plasma insulin concentration] decreased from  $41 \pm 7$  to  $25 \pm 3$  mg/kg fat-free mass (FFM)·min  $\times$   $\mu$ U/ml;  $P < 0.05$ ) and suppression of EGP during

the first insulin clamp step ( $1.1 \pm 0.1$  to  $0.6 \pm 0.2$  mg/kg FFM·min;  $P < 0.05$ ) improved after pioglitazone treatment. The total body glucose MCR during the first and second insulin clamp steps increased after pioglitazone treatment [first MCR,  $3.5 \pm 0.5$  to  $4.4 \pm 0.4$  ml/kg FFM·min ( $P < 0.05$ ); second MCR,  $8.7 \pm 1.0$  to  $11.3 \pm 1.1$  ml/kg FFM·min ( $P < 0.01$ )]. The improvement in hepatic and peripheral tissue insulin sensitivity occurred despite increases in body weight ( $82 \pm 4$  to  $85 \pm 4$  kg;  $P < 0.05$ ) and fat mass ( $27 \pm 2$  to  $30 \pm 3$  kg;  $P < 0.05$ ). After pioglitazone treatment, sc fat area at L4–5 ( $301 \pm 44$  to  $342 \pm 44$  cm<sup>2</sup>;  $P < 0.01$ ) increased, whereas visceral fat area at L4–5 ( $144 \pm 13$  to  $131 \pm 16$  cm<sup>2</sup>;  $P < 0.05$ ) and the ratio of visceral to sc fat ( $0.59 \pm 0.08$  to  $0.44 \pm 0.06$ ;  $P < 0.01$ ) decreased. In the postabsorptive state hepatic insulin resistance (basal EGP  $\times$  basal immunoreactive insulin) correlated positively with visceral fat area ( $r = 0.55$ ;  $P < 0.01$ ). The glucose MCRs during the first ( $r = -0.45$ ;  $P < 0.05$ ) and second ( $r = -0.44$ ;  $P < 0.05$ ) insulin clamp steps were negatively correlated with the visceral fat area. These results demonstrate that a shift of fat distribution from visceral to sc adipose depots after pioglitazone treatment is associated with improvements in hepatic and peripheral tissue sensitivity to insulin. (*J Clin Endocrinol Metab* 87: 2784–2791, 2002)

THIAZOLIDINEDIONES, a new class of insulin-sensitizing agents, have recently been introduced for the treatment of patients with type 2 diabetic mellitus. Early studies showed that troglitazone ameliorates insulin resistance and improves hyperglycemia, hyperinsulinemia, and dyslipidemia in type 2 diabetic patients (1–5). Although the precise mechanism of action of the thiazolidinediones remains to be determined, their glucose-lowering effect seems to depend on the presence of insulin (6). It is known that thiazolidinediones activate specific receptors, termed PPAR $\gamma$  (6, 7). PPAR $\gamma$  activation causes preadipocytes to differentiate into mature fat cells and causes the induction of key enzymes involved in lipogenesis (6, 8, 9). Consistent with these observations, clinical studies have demonstrated that thiazolidinedione therapy in type 2 diabetic patients is associated with weight gain, yet glycemic control improves (2, 5, 10, 11). The increase in body weight is positively related to the reduction in hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) (12). These results sug-

gest that the improvement in glucose homeostasis after thiazolidinedione treatment may in some way be related to an alteration in fat metabolism and/or fat topography. With respect to the later, Adams *et al.* (13) reported that thiazolidinediones promote the differentiation of preadipocytes into mature fat cells in sc, but not visceral, fat depots in humans. Numerous studies have shown that increased visceral fat is associated with insulin resistance and the development of macrovascular complications (14–16). Several recent studies have demonstrated that the thiazolidinedione, troglitazone, decreases visceral fat content in type 2 diabetic patients (10, 17), but no previous study has examined whether the alterations in abdominal fat distribution after thiazolidinedione treatment are related to the improvement in glycemic control and/or insulin sensitivity.

In the present study we have evaluated the effect of pioglitazone therapy on glucose tolerance, insulin secretion, hepatic and peripheral tissue sensitivity to insulin, plasma lipid levels, and abdominal fat distribution in type 2 diabetic individuals. To the best of our knowledge, this represents the first study that has examined the relationship between changes in abdominal fat distribution and glucose homeostasis/insulin sensitivity after thiazolidinedione treatment in type 2 diabetic subjects.

Abbreviations: EGP, Endogenous glucose production; FFM, fat-free mass; FPG, fasting plasma glucose concentration; HbA<sub>1c</sub>, hemoglobin A<sub>1c</sub>; HDL, high density lipoprotein; LDL, low density lipoprotein; MRI, magnetic resonance imaging; OGTT, oral glucose tolerance test; Ra, rate of endogenous glucose appearance; TGD, total glucose disposal.

## Subjects and Methods

### Subjects

Thirteen patients with type 2 diabetes mellitus (nine men and four women; age,  $52 \pm 3$  yr; five Caucasian, six Mexican-American, and two African-American; duration of diabetes,  $5 \pm 1$  yr) were recruited from the out-patient clinic of the Texas Diabetes Institute. Seven subjects had been taking a stable dose of sulfonylurea drugs for at least 3 months before our study. Six subjects were treated with diet alone. Patients who had previously received insulin, metformin, another thiazolidinedione, or acarbose were excluded. Entry criteria included age from 30–70 yr, body mass index less than  $36 \text{ kg/m}^2$ , stable body weight for at least 3 months before the study, and fasting plasma glucose concentration (FPG) between 140–240 mg/dl. All patients were in good general health without evidence of cardiac, hepatic, renal, or other chronic diseases, as determined by history, physical examination, screening blood tests, and urinalysis. No subject participated in any heavy exercise, and no subject was taking any medication known to affect glucose metabolism. All subjects gave signed voluntary, informed consent before participation. The institutional review board of the University of Texas Health Science Center (San Antonio, TX) approved the protocol.

### Study design

Three weeks before the study all subjects met with a dietician and were instructed to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein. During this period the FPG was measured at weekly intervals and varied by less than 5% in each subject. Blood pressure was measured during each visit, and  $\text{HbA}_{1c}$  and fasting plasma lipids were determined twice during this period. During the week before the start of pioglitazone treatment, all subjects received 1) a 75-g oral glucose tolerance test (OGTT), 2) measurement of fat-free mass (FFM) and fat mass using an iv bolus of  $^3\text{H}_2\text{O}$ , 3) quantitation of sc and visceral abdominal fat using nuclear magnetic resonance imaging (MRI), and 4) a two-step euglycemic insulin clamp study in combination with tritiated glucose and indirect calorimetry to examine hepatic and peripheral tissue sensitivity to insulin. All studies were performed at 0800 h after a 10- to 12-h overnight fast. Sulfonylurea-treated subjects did not take sulfonylurea on the day of the study.

After completion of these studies, subjects were started on pioglitazone (45 mg/d) for 16 wk. During the pioglitazone treatment period, subjects returned to the Clinical Research Center of the Texas Diabetes Institute at 0800 h every 2 wk for measurement of FPG, body weight, and blood pressure. Subjects refrained from eating or drinking anything except water after 2200 h on the night before each visit. On each visit dietary adherence was reinforced. Fasting plasma lipids [total cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol, and low density (LDL) lipoprotein cholesterol] were measured monthly.  $\text{HbA}_{1c}$  was measured twice during the last week of pioglitazone treatment.

### 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 zero each subject ingested 75 g glucose in 300 ml orange-flavored water, and plasma glucose, FFA, insulin and C peptide were measured at 15-min intervals for 2 h. At time zero a 100- $\mu\text{Ci}$  bolus of  $^3\text{H}_2\text{O}$  was given iv, and plasma tritiated water radioactivity was determined at 90, 105, and 120 min for calculation of FFM and fat mass.

### Abdominal fat distribution

Visceral and sc fat area were measured by MRI, using previously described imaging procedures (18). Briefly, images were acquired on a 1.9 T Elscint Prestige MRI system using a T1-weighted spin echo pulse sequence with a TR of 500 msec and a TE of less than 20 msec. A sagittal localizing image was used to center transverse sections on the line through the space between L4 and L5. Ten 5.0-mm thick sections were acquired with a gap of 1.0 mm to prevent signal cross-over from adjacent sections. The field of view ranged from 30–50 cm, depending on body size. Phase encoding was in the antero-posterior direction to minimize the effects of motion-induced phase artifacts that might otherwise be distributed laterally through a large portion of the abdomen. The field

of view was adjusted for body size to ensure 2-mm pixel spacing. Signal averaging (four signals averaged) were used to reduce the effects of motion-related artifacts. Additionally, respiratory gating was used to combat motion-induced artifacts and to reduce the blurring of fat boundaries in the anterior region of the abdomen. Images were processed using Alice software (Perceptive Systems, Inc., Boulder, CO) to determine abdominal sc and visceral fat areas. The sc fat area was analyzed by selecting the outer and inner margins of sc adipose tissue as region of interests from the cross-sectional images, and counting the number of pixels between the outer and inner margins of sc adipose tissue. The visceral (intraabdominal) fat area was determined using histograms specific to the visceral regions. The histograms were summed over a range of pixel values designated as fat by fitting two normal analysis distribution curves to them.

### Hyperinsulinemic euglycemic clamp

Insulin sensitivity was assessed with a two-step euglycemic insulin clamp, as previously described (19). Upon arrival (0800 h) at the Clinical Research Center, blood for measurement of FPG was obtained. A primed ( $25 \mu\text{Ci} \times \text{FPG}/100$ )-continuous ( $0.25 \mu\text{Ci}/\text{min}$ ) infusion of [ $^3\text{H}$ ]glucose was started at  $-180$  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 ( $60^\circ\text{C}$ ). Baseline arterialized venous blood samples for determination of plasma [ $^3\text{H}$ ]glucose radioactivity, and plasma glucose and insulin concentrations were drawn at  $-30$ ,  $-20$ ,  $-10$ ,  $-5$ , and 0 min. At time zero a primed-continuous infusion of human regular insulin (Novolin, Novo Nordisk Pharmaceuticals, Princeton, NJ) was started at a rate of  $40 \text{ mU}/\text{min}\cdot\text{m}^2$  body surface area and continued for 90 min. At 90 min the insulin space was reprimed, and the insulin infusion rate was increased to  $160 \text{ mU}/\text{min}\cdot\text{m}^2$  for an additional 90 min. After initiation of the insulin infusion, the plasma glucose concentration was allowed to drop spontaneously until it reached 90–100 mg/dl, at which level it was maintained by appropriately adjusting a variable infusion of 20% dextrose (19). Throughout the insulin clamp, blood samples for determination of plasma glucose were drawn every 5 min, and blood samples for determination of plasma insulin and [ $^3\text{H}$ ]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, as previously described (20).

### Assays

Plasma glucose was measured at bedside using the glucose oxidase method (Glucose Analyzer 2, Beckman Coulter, Inc., Fullerton, CA). Plasma insulin (Diagnostic Products, Los Angeles, CA) and C peptide (Diagnostics Systems Laboratories, Inc., Webster, TX) concentrations were measured by RIA.  $\text{HbA}_{1c}$  was measured by affinity chromatography (Biochemical Methodology, Drower 4350, Isolab, Akron, OH). Plasma FFA was measured by an enzymatic calorimetric quantification (Wako Chemicals, Neuss, Germany). Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were measured enzymatically (Roche Molecular Biochemicals, Indianapolis, IN) on a Hitachi 704 autoanalyzer. LDL cholesterol was calculated from the Friedewald equation. Tritiated glucose specific activity was determined on deproteinized plasma samples.

### Calculations

The effects of pioglitazone on all metabolic parameters and measures of fat mass/topography were similar both qualitatively and quantitatively in diet-treated and sulfonylurea-treated patients. Therefore, a combined analysis with all 13 diabetic patients is presented.

In the steady state, postabsorptive conditions the rate of endogenous glucose appearance ( $R_a$ ) is calculated as the [ $^3\text{H}$ ]glucose infusion rate (disintegrations per minute) divided by the steady state plasma [ $^3\text{H}$ ]glucose specific activity (disintegrations per minute). During the insulin clamp, nonsteady conditions prevailed, and the  $R_a$  was calculated from Steele's equation (21). Endogenous glucose production (EGP) was calculated as:  $\text{EGP} = R_a - \text{the glucose infusion rate}$ . Total glucose disposal (TGD) equals the sum of EGP plus the glucose infusion rate. The

total body glucose MCR equals the TGD divided by the plasma glucose concentration, where the 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 by indirect calorimetry, using formulas described previously (20). 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 plasma  $^3\text{H}_2\text{O}$  radioactivity at 90, 105, and 120 min after the iv bolus of  $^3\text{H}_2\text{O}$ . Plasma  $^3\text{H}_2\text{O}$  specific activity was calculated assuming that plasma water represents 93% of the total plasma volume. FFM was calculated by dividing total body water by 0.73 (22).

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 the duration of the OGTT (120 min).

### Statistical analysis

Statistics were performed with StatView for Windows, version 5.0 (SAS Institute, Inc., Cary, NC). Values before and after treatment were compared using paired *t* test. Linear regression analysis was used to examine the relationships between hepatic/peripheral insulin sensitivity and abdominal/sc fat area before and after pioglitazone treatment. All data are presented as the mean  $\pm$  SE. *P* value less than 0.05 was considered statistically significant.

## Results

### Body weight and fat distribution (Table 1)

After 16 wk of pioglitazone treatment, body weight, body mass index, and fat mass all increased significantly. The increase in fat mass (3.0 kg) precisely equaled the increase in body weight (3.0 kg). No change in lean body mass was observed during pioglitazone treatment. The increase in body weight was inversely related to the decrease in HbA<sub>1c</sub> ( $r = -0.70$ ;  $P < 0.01$ ). Pioglitazone treatment was associated with a significant increase in sc fat area and a significant decrease in visceral fat area. As a result, the visceral to sc fat ratio decreased significantly (Table 1). Changes in sc and

visceral fat in a typical diabetic patient are shown in Fig. 1. Plasma HbA<sub>1c</sub> and FPG decreased significantly after pioglitazone treatment. The fasting plasma insulin concentration tended to decrease, whereas the fasting plasma C peptide concentration declined significantly. Pioglitazone treatment decreased the mean plasma glucose concentration during the OGTT without a change in the mean plasma insulin or C peptide concentrations. The incremental area under the plasma glucose concentration curve ( $11,813 \pm 516$  vs.  $10,492 \pm 542$  mg/dl·min) declined significantly ( $P < 0.05$ ) after pioglitazone treatment, indicating a beneficial effect on postprandial hyperglycemia. Consistent with this effect on postprandial hyperglycemia, the decrement in the mean plasma glucose concentration ( $63 \pm 10$ ) during the OGTT was significantly greater ( $P < 0.05$ ) than the decrement in the FPG concentration ( $40 \pm 6$ ).

### Plasma lipids

Fasting plasma triglyceride ( $P < 0.01$ ) and FFA ( $P < 0.05$ ) concentrations decreased significantly after pioglitazone treatment. Total cholesterol, HDL cholesterol, and LDL cholesterol concentrations did not change.

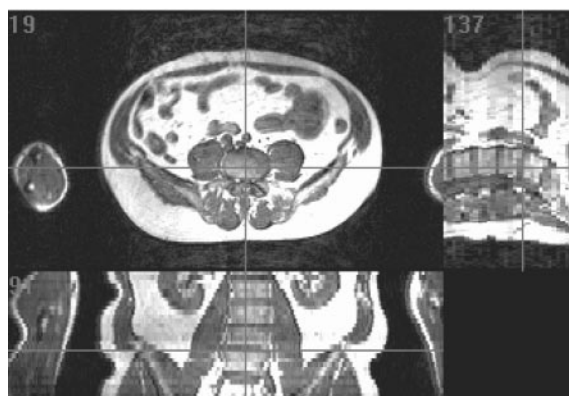
### Euglycemic insulin clamp

**Plasma glucose and insulin.** During the insulin clamp performed before pioglitazone, the steady state plasma insulin concentrations during the first ( $63 \pm 7$   $\mu\text{U}/\text{ml}$ ) and second ( $331 \pm 24$   $\mu\text{U}/\text{ml}$ ) steps were similar to those during the insulin clamp performed after pioglitazone treatment (first step,  $64 \pm 5$   $\mu\text{U}/\text{ml}$ ; second step,  $310 \pm 12$   $\mu\text{U}/\text{ml}$ ). During the initial insulin clamp study the mean plasma glucose concentrations during the first and second steps were  $106 \pm 6$  and  $90 \pm 1$  mg/dl. During the first and second steps of the insulin clamp performed after pioglitazone treatment, the

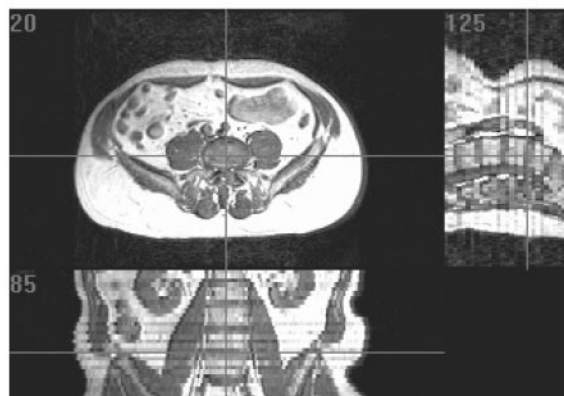
**TABLE 1.** Body weight and fat distribution, parameters of glycemic control, and plasma lipids before and after pioglitazone treatment for 16 wk

|  | Before          | After           | <i>P</i> value |
|--|-----------------|-----------------|----------------|
| BW (kg)  | 82 $\pm$ 4      | 85 $\pm$ 5      | 0.010          |
| BMI (kg/m <sup>2</sup> )                           | 29.0 $\pm$ 1.1  | 30.2 $\pm$ 1.1  | 0.009          |
| Fat mass (kg)                                      | 27 $\pm$ 2      | 30 $\pm$ 3      | 0.001          |
| Fat-free mass (kg)                                 | 55 $\pm$ 3      | 55 $\pm$ 3      |                |
| Fasting plasma glucose (mg/dl)                     | 179 $\pm$ 10    | 140 $\pm$ 10    | <0.001         |
| HbA <sub>1c</sub> (%)                              | 8.6 $\pm$ 0.4   | 7.2 $\pm$ 0.5   | 0.003          |
| Fasting plasma insulin ( $\mu\text{U}/\text{ml}$ ) | 15 $\pm$ 2      | 11 $\pm$ 2      |                |
| Fasting plasma C peptide (ng/ml)                   | 2.4 $\pm$ 0.2   | 1.7 $\pm$ 0.3   | 0.045          |
| Total cholesterol (mg/dl)                          | 164 $\pm$ 10    | 157 $\pm$ 11    |                |
| LDL cholesterol (mg/dl)                            | 101 $\pm$ 9     | 102 $\pm$ 10    |                |
| HDL cholesterol (mg/dl)                            | 34 $\pm$ 2      | 35 $\pm$ 2      |                |
| Triglycerides (mg/dl)                              | 144 $\pm$ 17    | 100 $\pm$ 10    | 0.002          |
| FFA ( $\mu\text{Eq}/\text{liter}$ )                | 674 $\pm$ 38    | 569 $\pm$ 31    | 0.010          |
| 75-g OGTT  |                 |                 |                |
| Mean plasma glucose (mg/dl)                        | 295 $\pm$ 13    | 233 $\pm$ 14    | <0.001         |
| Mean plasma insulin ( $\mu\text{U}/\text{ml}$ )    | 32 $\pm$ 8      | 32 $\pm$ 7      |                |
| Mean plasma C peptide (ng/ml)                      | 3.8 $\pm$ 0.6   | 3.6 $\pm$ 0.5   |                |
| Mean plasma FFA ( $\mu\text{Eq}/\text{liter}$ )    | 539 $\pm$ 20    | 396 $\pm$ 26    | <0.001         |
| Body fat distribution                              |                 |                 |                |
| Subcutaneous fat area (cm <sup>2</sup> )           | 301 $\pm$ 44    | 342 $\pm$ 44    | <0.001         |
| Visceral fat area (cm <sup>2</sup> )               | 144 $\pm$ 13    | 131 $\pm$ 16    | 0.040          |
| Visceral to sc fat ratio                           | 0.59 $\pm$ 0.08 | 0.44 $\pm$ 0.06 | 0.005          |

Data are the mean  $\pm$  SEM (*n* = 13).

**Case: Male/59 years old****Baseline**

**Subcutaneous Fat Area: 144.3 cm<sup>2</sup>**  
**Visceral Fat Area: 140.0 cm<sup>2</sup>**  
**Body Weight: 67.4 kg**  
**FPG: 184 mg/dl**  
**HbA<sub>1c</sub>: 7.3 %**

**Pioglitazone for 16 weeks**

**Subcutaneous Fat Area: 204.7 cm<sup>2</sup>**  
**Visceral Fat Area: 105.1 cm<sup>2</sup>**  
**Body Weight: 69.2 kg**  
**FPG: 117 mg/dl**  
**HbA<sub>1c</sub>: 6.5 %**

**FIG. 1.** MRI study demonstrating the change in visceral and sc abdominal fat area in a typical pioglitazone-treated patient. Each picture shows a transverse cross-sectional view (*left upper*), sagittal view (*right upper*), and coronal view (*left lower*) before and after pioglitazone treatment. The transverse cross-sectional view at the L4–5 vertebral level was used in the measurement of sc and visceral fat areas.

**TABLE 2.** Hepatic and whole body glucose metabolism during the two-step insulin clamp (insulin infusion rates: 40 and 160 mU/m<sup>2</sup> · min) performed before and after 16 wk of pioglitazone treatment

|   | 16-wk treatment |              | P value |
|---|-----------------|--------------|---------|
|   | Before          | After        |         |
| Basal (–30 to 0 min)  |                 |              |         |
| Plasma glucose (mg/dl)  | 155 ± 10        | 127 ± 10     | 0.004   |
| Plasma insulin (μU/ml)  | 15 ± 2          | 10 ± 1       | 0.013   |
| Endogenous glucose production (mg/kg FFM · min)                   | 2.83 ± 0.17     | 2.66 ± 0.07  |         |
| Hepatic insulin resistance (mg/kg FFM · min × μU/ml) <sup>a</sup> | 41 ± 7          | 25 ± 3       | 0.011   |
| 1st insulin clamp step (60–90 min)                                |                 |              |         |
| Endogenous glucose production (mg/kg FFM · min)                   | 1.07 ± 0.14     | 0.63 ± 0.16  | 0.025   |
| Total body glucose disposal (mg/kg FFM · min)                     | 3.57 ± 0.41     | 4.29 ± 0.39  | 0.091   |
| Total body glucose clearance (ml/kg FFM · min)                    | 3.51 ± 0.47     | 4.38 ± 0.44  | 0.042   |
| 2nd insulin clamp step (150–180 min)                              |                 |              |         |
| Endogenous glucose production (mg/kg FFM · min)                   | 0.44 ± 0.14     | 0.34 ± 0.09  |         |
| Total body glucose disposal (mg/kg FFM · min)                     | 7.80 ± 0.91     | 10.30 ± 1.00 | 0.001   |
| Total body glucose clearance (ml/kg FFM · min)                    | 8.65 ± 0.99     | 11.31 ± 1.11 | 0.004   |

Data are the mean ± SEM (n = 13).

<sup>a</sup> Product of EGP and fasting plasma insulin concentration in the postabsorptive state.

mean plasma glucose concentrations were 101 ± 6 and 92 ± 1 mg/dl.

**EGP.** The basal rate of EGP during the initial insulin clamp (2.83 ± 0.17 mg/kg FFM·min) was slightly, although not significantly, higher than the basal rate of EGP (2.66 ± 0.07) after 16 wk of pioglitazone treatment. Under postabsorptive conditions the majority (85–90%) of EGP is derived from the liver (23). Therefore, the product of the basal rate of EGP and the simultaneously measured fasting plasma insulin concentration provides a measure of hepatic insulin resistance. After pioglitazone treatment, hepatic insulin resistance during

the postabsorptive state declined by 61% from 41 ± 7 to 25 ± 3 mg/kg FFM·min × μU/ml ( $P < 0.05$ ; Table 2 and Fig. 2).

**Glucose metabolism during insulin clamp.** During the first step of the insulin clamp, the suppression of EGP was 60% greater after pioglitazone treatment compared with that during the baseline study performed before pioglitazone treatment ( $P = 0.05$ ; Table 2). During the second insulin clamp step, the suppression of EGP was similar in the pre- and postpioglitazone studies (Table 2). Total body glucose disposal during the first insulin clamp step tended to be increased ( $P = 0.09$ ) after pioglitazone treatment, and total body glucose MCR

rose significantly ( $P < 0.05$ ; Table 2 and Fig. 3). Total body glucose disposal during the second insulin clamp step increased by 32% ( $P < 0.001$ ) after pioglitazone treatment, and this was paralleled by a similar rise in the total body glucose MCR ( $P < 0.01$ ; Table 2 and Fig. 3). Nonoxidative glucose disposal during the second step insulin clamp increased significantly after pioglitazone treatment ( $4.36 \pm 0.82$  to  $6.45 \pm 0.81$  mg/kg FFM·min;  $P < 0.01$ ); there was no change in oxidative glucose disposal ( $3.48 \pm 0.22$  vs.  $3.90 \pm 0.25$  mg/kg FFM·min). During the first step insulin clamp, nonoxidative glucose disposal ( $1.26 \pm 0.37$  vs.  $1.96 \pm 0.40$  mg/kg FFM·min) rose slightly, but not significantly ( $P = 0.08$ ), after pioglitazone treatment, whereas oxidative glucose disposal remained unchanged ( $2.31 \pm 0.17$  vs.  $2.36 \pm 0.22$  mg/kg FFM·min). Basal and insulin-mediated suppressions of lipid

oxidation during the first and second insulin clamp steps were similar before ( $1.01 \pm 0.06$  to  $0.72 \pm 0.06$  to  $0.43 \pm 0.08$  mg/kg FFM·min) and after ( $1.06 \pm 0.11$  to  $0.74 \pm 0.13$  to  $0.33 \pm 0.13$  mg/kg FFM·min) pioglitazone treatment.

#### Regression analysis

Visceral fat area correlated positively with hepatic insulin resistance ( $r = 0.55$ ;  $P < 0.01$ ); sc fat area did not show any correlation with hepatic insulin resistance (Fig. 4). Visceral fat area was inversely correlated with the total body glucose MCR during both the first step ( $r = -0.45$ ;  $P < 0.05$ ) and the second step ( $r = -0.44$ ;  $P < 0.05$ ) of the insulin clamp (Fig. 5); the sc fat area did not correlate with the glucose MCR (Fig. 5).

#### Discussion

The beneficial effects of the thiazolidinediones on glucose metabolism are believed to be mediated by their binding to the nuclear receptor PPAR $\gamma$  (6). PPAR $\gamma$  receptors are most abundant in adipocytes and are present in low concentrations in muscle (24). PPAR $\gamma$  activation results in stimulation of adipogenesis (6, 8, 9, 25) and gene transcription of key enzymes involved in lipogenesis (6, 8). There is significant variability in the adipose tissue distribution of PPAR $\gamma$ . Thus, Lefebvre *et al.* (26) have demonstrated that mRNA levels of PPAR $\gamma$  in man are significantly lower in visceral (omental) fat compared with sc fat, especially in individuals with a body mass index less than 30 kg/m<sup>2</sup>. When thiazolidinediones are administered to animals (8) and humans (2, 5, 10), weight gain is commonly observed. Nonetheless, glucose homeostasis and insulin sensitivity improve (2, 5, 10). Recent studies have demonstrated that the weight gain is associated with an increase in sc adipose tissue and a concomitant decrease in visceral fat content (10, 17). This fat redistribution is explained by PPAR $\gamma$  agonist-induced remodeling of abdominal fat tissue, characterized by differentiation of preadipocytes into small fat cells in sc fat depots (8, 13, 25) and apoptosis of differentiated large adipocytes (hypertrophic adipocytes) in visceral and/or sc fat depots (25, 27). These observations may have clinical relevance to the mechanism of action of thiazolidinediones, as in humans visceral adiposity is associated with insulin resistance (14–16), and in animals surgical removal of visceral fat enhances insulin sensitivity (28).

After 16 wk of pioglitazone treatment, all 13 diabetic subjects gained weight, which was accounted for entirely by an increase in fat mass. Despite the weight gain, glycemic control improved, and the decline in HbA<sub>1c</sub> was correlated with the increase in body weight ( $r = -0.70$ ;  $P < 0.01$ ). The weight gain was associated with redistribution of fat from visceral to sc adipose depots. A strong correlation was noted between the visceral fat area and the hepatic insulin resistance index before and after pioglitazone treatment (Fig. 4). Previous studies have shown that visceral adipocytes are lipolytically more active than sc fat cells (29), and increased delivery of FFA into the portal circulation would be expected to augment hepatic gluconeogenesis (30, 31). Conversely, thiazolidinediones have been shown to be potent inhibitors of gluconeogenesis *in vitro* (32) and to suppress basal hepatic glucose production *in vivo* (1, 3). Consistent with this sce-

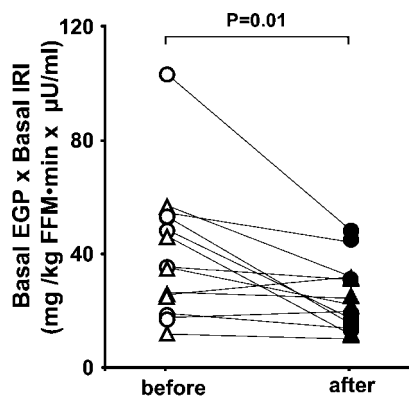


FIG. 2. Index of hepatic insulin resistance (basal EGP  $\times$  basal IRI) before and after 16 wk of pioglitazone treatment, where EGP is the endogenous glucose production (milligrams per kilogram FFM per minute) during the basal period, and IRI is the immunoreactive insulin concentration (microunits per milliliter). Sulfonyleurea-treated subjects are indicated by the circles, and diet-treated subjects by the triangles.

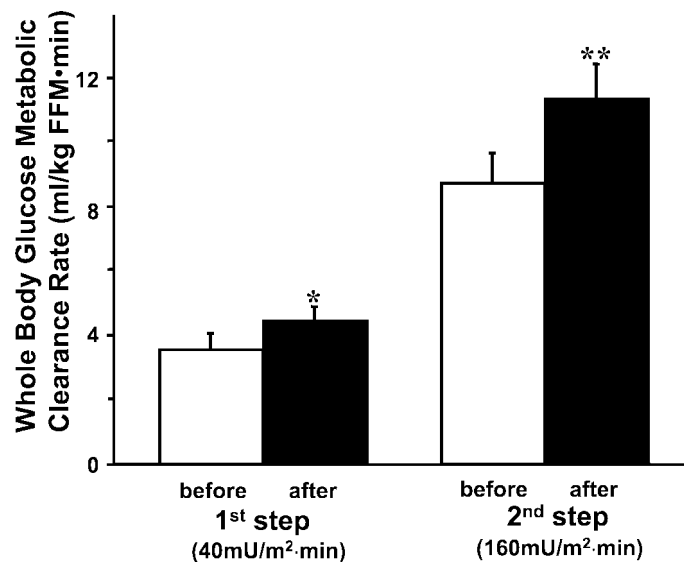


FIG. 3. Total body glucose MCR (milliliters per kilogram FFM per minute) during the first and second steps of the insulin clamp performed before and after 16 wk of pioglitazone treatment. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$  (before vs. after pioglitazone).

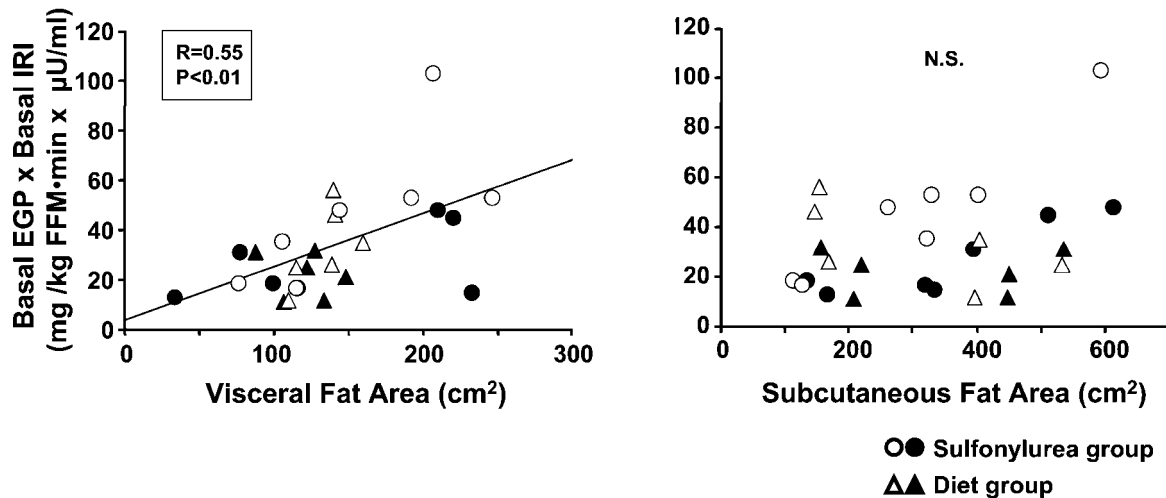


FIG. 4. Association between visceral fat area (*left*)/sc fat area (*right*) at the L4–5 vertebral level and hepatic insulin resistance (basal EGP  $\times$  basal IRI) before and after 16 wk of pioglitazone treatment combined. *Open symbols*, Before pioglitazone; *closed symbols*, after pioglitazone. Sulfonyleurea-treated subjects are indicated by the *circles*, and diet-treated subjects by the *triangles*.

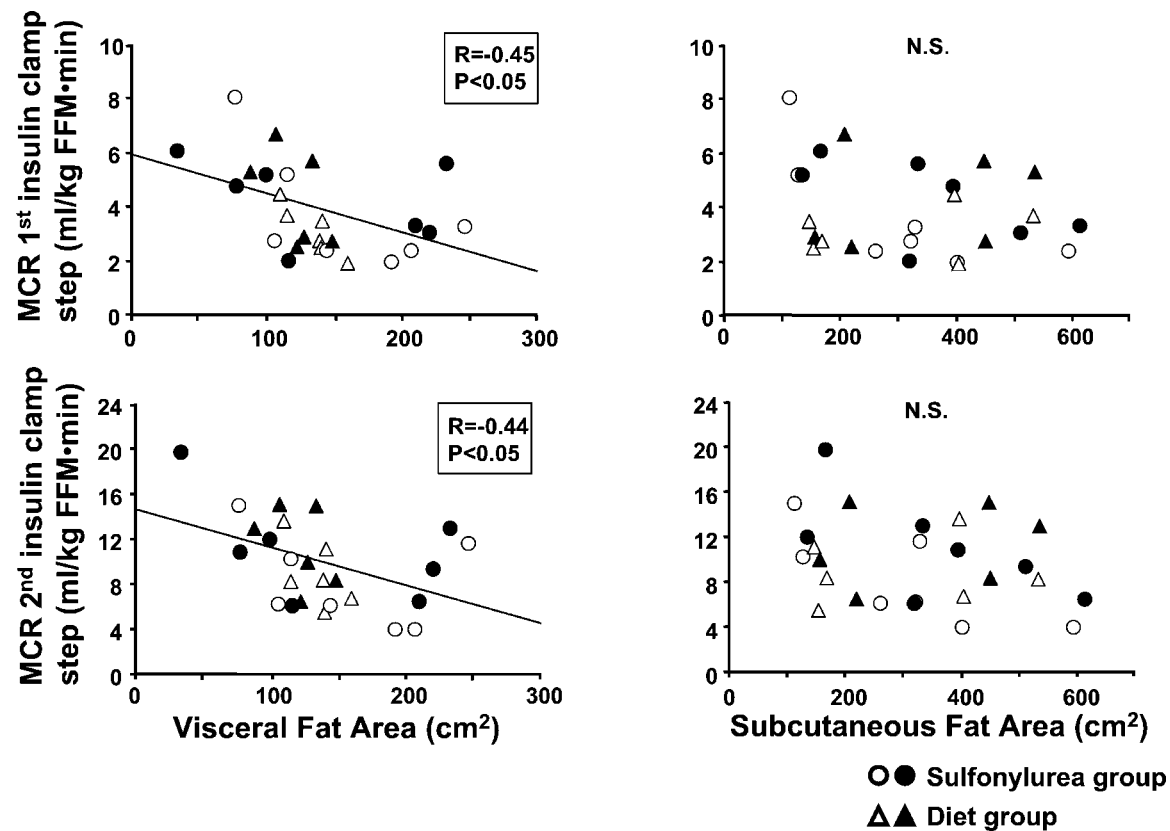


FIG. 5. Association between visceral fat area (*left*)/sc fat area (*right*) at L4–5 vertebral level and total body glucose MCR during the first and second steps of the insulin clamp performed before and after 16 wk of pioglitazone treatment. *Open symbols*, Before pioglitazone; *closed symbols*, after pioglitazone. Sulfonyleurea-treated subjects are indicated by the *circles*, and diet-treated subjects by the *triangles*.

nario, we noted a positive correlation ( $r = 0.44$ ;  $P < 0.05$ ) between the basal plasma FFA concentration and hepatic insulin resistance under postabsorptive conditions (Fig. 6).

The fasting plasma glucose concentration was strongly correlated with the basal rate of EGP both before ( $r = 0.71$ ;  $P < 0.01$ ) and after ( $r = 0.63$ ;  $P < 0.05$ ) pioglitazone treatment.

Although pioglitazone therapy was associated with a modest decline in EGP, this did not reach statistical significance, most likely because 1) the fasting plasma insulin concentration fell by 33% (15 to 10  $\mu\text{U/ml}$ ;  $P = 0.01$ ), and this would oppose the inhibitory action of pioglitazone on basal EGP; and 2) during the 3-h period required for tritiated glucose

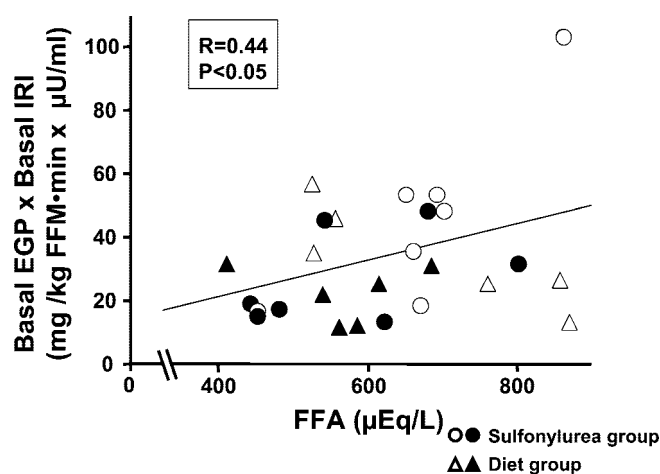


FIG. 6. Relationship between hepatic insulin resistance (basal EGP  $\times$  basal insulin concentration) and the fasting plasma FFA concentration in diabetic patients before (open symbols) and after (closed symbols) pioglitazone treatment. Sulfonyleurea-treated subjects are indicated by the circles, and diet-treated subjects by the triangles.

equilibration, the FPG declined to  $155 \pm 10$  mg/dl (initial study) and to  $127 \pm 10$  mg/dl (post-pioglitazone study) at the time ( $\sim 1100$ – $1130$  h) that EGP was measured. We previously showed that EGP does not increase until the FPG exceeds 140 mg/dl (33), and Reaven *et al.* (34) reported an even higher threshold, an FPG of approximately 160 mg/dl. That pioglitazone increased hepatic insulin sensitivity is documented by the enhanced suppression of EGP during the first insulin clamp step as well as the reduction in postabsorptive hepatic insulin resistance index.

All previous studies that examined insulin sensitivity after thiazolidinedione treatment in type 2 diabetic patients employed very high insulin infusion rates (80,120,300 mU/m<sup>2</sup>·min) that produced pharmacological plasma insulin concentrations (1, 3, 35, 36). In the present study we employed both physiological (40 mU/m<sup>2</sup>·min) and pharmacological (160 mU/m<sup>2</sup>·min) insulin infusion rates. Consistent with prior studies, we observed a 32% improvement in insulin sensitivity during the high dose insulin clamp. However, during the lower dose insulin clamp the improvements in total body glucose disposal (20%) and glucose metabolic clearance (22%) rates were more modest. During both the high and low dose insulin clamp studies, the whole body MCR of glucose was inversely correlated with the visceral fat area, but not with the sc fat area. Previous studies have shown that visceral adipocytes are lipolytically more active than sc fat cells (29). Pioglitazone, by decreasing the amount of visceral fat, could lead to a reduction in the plasma FFA concentration, as was observed in the present study. However, we failed to observe any correlation between either the reduction in fasting or post-OGTT plasma FFA concentration and the improvement in insulin-stimulated glucose MCR. Alternatively, the correlation between the glucose clearance rate and visceral fat area before and after pioglitazone treatment could be explained by the production by visceral adipocytes of some other (than FFA) circulating factor that induces insulin resistance in peripheral (muscle) tissue, such as TNF $\alpha$ , which was not measured in the present study (37, 38).

However, any decrease in TNF $\alpha$  production associated with reduced visceral fat would be offset by enhanced TNF $\alpha$  production by the increase in sc fat content (28). Even if the plasma TNF $\alpha$  concentration did not decline, one could not exclude a local paracrine effect of reduced tissue TNF $\alpha$  in muscle or liver. Lastly, a growing number of fat cell-secreted peptides have been described (39–41), which could contribute to the improvements in muscle and hepatic insulin sensitivity after the pioglitazone-induced changes in fat topography.

The increases in hepatic and peripheral tissue sensitivity to insulin after pioglitazone treatment were associated with a significant improvement in glucose homeostasis, as reflected by a 1.4% decrease in HbA<sub>1c</sub>. The reduction in fasting plasma glucose (39 mg/dl) was rather modest and cannot alone account for the decline in HbA<sub>1c</sub>. Consistent with these observations, the decrement in mean plasma glucose concentration ( $63 \pm 10$  mg/dl) during the OGTT was significantly greater ( $P < 0.05$ ) than the decrement in FPG ( $39 \pm 6$  mg/dl). The beneficial effect of pioglitazone on postprandial hyperglycemia is quite distinct from that of the sulfonyleureas (42) and metformin (43), which exert their primary effect on the FPG concentration. The factors responsible for the improvement in postprandial hyperglycemia are probably multifactorial. During the first step insulin clamp, suppression of hepatic glucose production was improved by 41% and correlated with the decline in postprandial hyperglycemia ( $r = 0.47$ ;  $P = 0.01$ ). Pioglitazone also enhanced peripheral tissue insulin sensitivity, but this was most marked during the high dose, pharmacological second insulin clamp step. During the lower, more physiological first insulin clamp step, the improvements in glucose clearance rate and total body glucose disposal rate were quite small, suggesting that factors in addition to enhanced peripheral tissue sensitivity to insulin must have contributed to the improvement in glucose homeostasis. Two mechanisms deserve consideration: 1) enhanced splanchnic (hepatic) glucose uptake, and 2) enhanced glucose-mediated glucose uptake or an enhanced effect of the combined action of hyperinsulinemia plus hyperglycemia to augment peripheral tissue (muscle) glucose uptake. The former can be evaluated with a dual isotopic tracer technique (44) or a combined oral glucose/insulin clamp technique (45). Insight about the effect of pioglitazone to enhance the combined effects of hyperinsulinemia plus hyperglycemia on whole body glucose disposal can be gained by using the plasma glucose and insulin concentrations during the OGTT to derive a composite index of whole body insulin sensitivity (46). Using this index, whole body insulin sensitivity, measured under hyperglycemic conditions, improved by 43% from  $2.70 \pm 0.35$  to  $3.87 \pm 0.50$  ( $P < 0.01$ ). This marked improvement in the whole body insulin sensitivity index during the OGTT stands in contrast to the small improvement demonstrated during the first insulin clamp step.

In summary, the present results demonstrate for the first time that the pioglitazone-induced decrease in visceral fat is associated with improvements in both hepatic and peripheral tissue sensitivity to insulin. Changes in insulin sensitivity after pioglitazone are unrelated to the increase in sc fat content. Despite a consistent and significant increase in body weight and total body fat mass, glycemic control and both

hepatic and peripheral tissue sensitivity to insulin improved after pioglitazone treatment.

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Address all correspondence and requests for reprints to: Ralph A DeFronzo, M.D., Diabetes Division, University of Texas Health Science Center, Room 3.380S, 7703 Floyd Curl Drive, San Antonio, Texas 78229-3900. E-mail: albarado@uthscsa.edu.

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