

Dose-Response Effect of Pioglitazone on Insulin Sensitivity and Insulin Secretion in Type 2 Diabetes

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OBJECTIVE — To investigate the dose-response effects of pioglitazone on glycemic control, insulin sensitivity, and insulin secretion in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 58 diet-treated patients with type 2 diabetes (aged 54 ± 1 years; 34 men and 24 women; BMI 31.5 ± 0.6 kg/m²) were randomly assigned to receive placebo ($n = 11$) or 7.5 mg ($n = 13$), 15 mg ($n = 12$), 30 mg ($n = 11$), or 45 mg ($n = 11$) of pioglitazone per day for 26 weeks. Before and after 26 weeks, subjects underwent a 75-g oral glucose tolerance test (OGTT).

RESULTS — Patients treated with 7.5 or 15 mg/day of pioglitazone had no change in fasting plasma glucose (FPG) and fasting plasma insulin (FPI) concentrations or in plasma glucose (PG) and insulin concentrations during the OGTT. Patients treated with 30 and 45 mg/day of pioglitazone, respectively, had significant decreases from placebo in HbA_{1c} ($\Delta = -2.0$ and -2.9%), FPG ($\Delta = -66$ and -97 mg/dl), and mean PG during OGTT ($\Delta = -84$ and -107 mg/dl). Fasting plasma insulin decreased significantly in the 45-mg/day pioglitazone group, but the mean plasma insulin during the OGTT did not change. The insulinogenic index (Δ area under the curve [AUC] insulin/ Δ AUC glucose) during the OGTT increased significantly in the 30- and 45-mg/day pioglitazone groups (0.13 ± 0.03 to 0.27 ± 0.05 , $P < 0.05$). From the OGTT, we previously have derived a composite whole-body insulin sensitivity index (ISI) that correlates well with that measured directly with the insulin clamp technique. Whole-body ISI [$ISI = 10,000/\sqrt{(FPG \times FPI) \times (\overline{PG} \times \overline{PI})}$], where \overline{PG} and \overline{PI} equal mean plasma glucose and insulin concentrations during OGTT] increased significantly in patients treated with 30 mg (1.8 ± 0.3 to 2.5 ± 0.3 , $P < 0.05$) or 45 mg (1.6 ± 0.2 to 2.7 ± 0.6 , $P < 0.05$) per day of pioglitazone. In the basal state, the hepatic ISI [$k/(FPG \times FPI)/k/(FPG \times FPI)$], which agrees closely with that measured directly with tritiated glucose, increased in patients treated with 30 mg (0.13 ± 0.02 to 0.21 ± 0.03 , $P < 0.05$) and 45 mg (0.11 ± 0.02 to 0.24 ± 0.06 , $P < 0.05$) per day of pioglitazone. Significant correlations between the dose of pioglitazone and the changes in HbA_{1c} ($r = -0.58$), FPG ($r = -0.47$), mean PG during the OGTT ($r = -0.46$), insulinogenic index ($r = 0.34$), hepatic ISI ($r = 0.44$), and whole-body ISI ($r = 0.36$) were observed.

CONCLUSIONS — Pioglitazone improves glycemic control through the dose-dependent enhancement of β -cell function and improved whole-body and hepatic insulin sensitivity.

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Abbreviations: AUC, area under the curve; FPG, fasting plasma glucose; FPI, fasting plasma insulin; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; PG, plasma glucose; PPAR γ , peroxisome proliferator-activated receptor- γ .

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Thiazolidinediones, a new class of insulin-sensitizing agents, recently have been introduced for the treatment of patients with type 2 diabetes. Early studies showed that troglitazone ameliorates insulin resistance and improves hyperglycemia and hyperinsulinemia in type 2 diabetes (1,2). Thiazolidinediones activate a specific nuclear receptor, termed peroxisome proliferator-activated receptor- γ (PPAR γ) (3), causing preadipocytes to differentiate into mature fat cells and inducing key lipogenic enzymes (3,4). A close relationship exists between the ability of various thiazolidinediones to activate PPAR γ and their hypoglycemic action (5). 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 (6). Recently, Aronoff et al. (7) demonstrated a dose-dependent beneficial effect of pioglitazone monotherapy on glycemic control in type 2 diabetic patients in a large-scale multicenter trial. However, only one previous study has examined the effect of pioglitazone on insulin sensitivity in diabetic humans (8), and no clinical study has examined the dose-dependent effect of pioglitazone on insulin sensitivity in vivo. Previously, we derived a composite index of whole-body insulin sensitivity during 75-g oral glucose tolerance testing (OGTT) and demonstrated that this index correlates well with that measured directly with the insulin clamp technique (9). Using this insulin sensitivity index (ISI), we have examined the dose-dependent effect of pioglitazone on whole-body insulin sensitivity and insulin secretion in 58 patients with type 2 diabetes after 26 weeks of treatment with pioglitazone or placebo. This analysis provides important new information about the mechanism, as well as the dose-response characteristics of pioglitazone, in the treatment of patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study design

A total of 58 patients with type 2 diabetes underwent 75-g OGTT before and after 26 weeks of pioglitazone therapy in a randomized, double-blind, placebo-control, multicenter clinical trial. To be eligible, patients were required to have HbA_{1c} \geq 7.0%, fasting plasma glucose (FPG) \geq 140 mg/dl, and fasting C-peptide $>$ 1 ng/ml. Patients who used insulin or who had unstable proliferative retinopathy, impaired liver function (aspartate aminotransferase or alanine aminotransferase $>$ 2.5 \times upper limit of normal), impaired kidney function (serum creatinine $>$ 1.8 mg/dl), or anemia were excluded. Patients taking previous antidiabetic therapy (sulfonylureas or metformin) underwent a 6- to 8-week single-blind washout period before the baseline OGTT was performed. After the washout period, only patients with HbA_{1c} \geq 7.0% were enrolled. Patients were randomized to one of five parallel treatment groups: pioglitazone 7.5, 15, 30, or 45 mg/day or placebo. During the double-blind period, patients were seen every 2 weeks for the first 6 weeks and every 4 weeks for the remaining 20 weeks. At 26 weeks, all subjects underwent repeat 75-g OGTT. To minimize the confounding effect of weight loss on metabolic changes, no specific dietary modifications were recommended during the study.

Measurements

During the OGTT, plasma glucose (PG) concentration was measured at 0, 1, and 2 h by the hexokinase method (Hitachi 747-200 Analyzer; Roche, Indianapolis, IN). Plasma insulin concentration was measured at 0, 1, and 2 h during OGTT by an automated enzyme immunoassay (Tosoh AIA-1200; Tosoh Medicus, South San Francisco, CA). HbA_{1c} was measured by automated ion-exchange high-performance liquid chromatography (Bio-Rad Variant Analyzer; Bio-Rad Diagnostics, Hercules, CA). Fasting serum lipid levels (total cholesterol, HDL cholesterol, and triglycerides) were determined enzymatically (Hitachi 747 Analyzer; Roche, Indianapolis, IN). LDL cholesterol was calculated from the Friedewald equation.

Calculations

The whole-body ISI was determined from the OGTT as follows (9):

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

where FPI = fasting plasma insulin (μ U/ml), FPG = fasting plasma glucose (mg/dl), and $\overline{\text{PG}}$ and $\overline{\text{PI}}$ represent the mean plasma glucose and plasma insulin concentrations during OGTT. This index provides a composite measure of the combined effects of hyperinsulinemia plus hyperglycemia on muscle and hepatic glucose metabolism and is highly correlated with insulin sensitivity measured with the euglycemic insulin clamp technique (9). In the previous study (9), whole-body ISI was calculated from PG and insulin concentrations measured every 30 min during OGTT. In the present study, PG and insulin were measured every hour during the OGTT. Therefore, using the previously published data (9), we reanalyzed the relationship between insulin sensitivity obtained from euglycemic insulin clamp and ISI derived from PG and insulin concentrations at 0, 1, and 2 h during OGTT. A highly significant correlation between these parameters was observed in nondiabetic ($r = 0.74$, $P < 0.001$) and type 2 diabetic ($r = 0.67$, $P < 0.001$) individuals.

Hepatic insulin sensitivity can be estimated from the FPG and FPI as follows (9,10):

$$\frac{k}{\text{FPG} \times \text{FPI}}$$

This equation is mathematically equivalent to the reduced formula of the homeostasis model assessment (HOMA) (10), where $k = 22.5 \times 18$, and the index of hepatic insulin sensitivity correlates closely with that measured directly with tritiated glucose (9).

Statistical analysis

Statistical calculations were performed using StatView for Windows software (version 5.0; SAS Institute, Cary, NC). Values before and after treatment within each group were analyzed using paired Student's *t* test. Comparison between groups was performed using ANOVA with Bonferroni/Dunn post-hoc testing. Comparisons over time were made using

repeated measures ANOVA. Pearson's correlations between continuous variables were used as a measure of association. For the association between the five groups and changes in metabolic variables after treatment with pioglitazone or placebo, we also reevaluated the significance using Spearman's correlation coefficient by rank. All data are presented as the mean \pm SEM. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics, HbA_{1c}, glucose, insulin, and lipids

Fifty-eight patients were randomized to receive placebo ($n = 11$) or 7.5 ($n = 13$), 15 ($n = 12$), 30 ($n = 11$), or 45 ($n = 11$) mg/day of pioglitazone. At baseline, there were no significant differences in age, ethnicity, sex, BMI, HbA_{1c}, FPG, or insulin concentrations between the placebo and four pioglitazone-treated groups (Table 1). There tended to be more men than women in the pioglitazone-treated groups compared with the placebo group. Within the four pioglitazone-treated groups, the distribution of men and women was similar. It is known that men respond less well to thiazolidinediones than women because of their lower percentage of body fat (11). Plasma lipid levels were similar in the pioglitazone and placebo groups (Table 1).

After 26 weeks of treatment, there was a dose-dependent increase in body weight and BMI in the pioglitazone-treated groups (Table 1). The increments in body weight (4.5 ± 0.7 kg) and BMI (1.6 ± 0.3 kg/m²) were significantly greater ($P < 0.01$) in the 45-mg/day pioglitazone group than in the placebo group. Plasma HbA_{1c} and FPG also demonstrated a dose-dependent decrease, with significant decrements in HbA_{1c} in the 15-, 30-, and 45-mg/day pioglitazone groups ($P < 0.05$ – 0.001 versus placebo) (Table 1). The increase in HbA_{1c} in the placebo group most likely reflects the lack of equilibration of HbA_{1c} during the 6- to 8-week washout period and, to a lesser extent, the progressive nature of type 2 diabetes. A highly significant inverse relationship was observed between change in body weight and change in HbA_{1c} ($n = 58$, $r = -0.527$, $P < 0.0001$). Fasting plasma insulin concentration decreased slightly in all pioglitazone-treated groups,

Table 1—Changes in metabolic and laboratory parameters at 26 weeks compared with baseline

	Placebo	Pioglitazone			
		7.5 mg/day	15 mg/day	30 mg/day	45 mg/day
Men/Women	3/8	10/3	8/4	8/3	5/6
Ethnicity (C/AA/MA/A/others)	9/1/1/0/0	10/1/0/2/0	10/1/1/0/0	6/0/4/0/1	7/1/2/0/1
Age (years)	58 ± 3	51 ± 3	57 ± 4	51 ± 2	55 ± 2
Weight (kg)					
Baseline	90 ± 4	93 ± 5	93 ± 5	97 ± 4	86 ± 3
Week 26	90 ± 4	93 ± 5	95 ± 7*	100 ± 4*	91 ± 3†
Change	-0.4 ± 1.4	0.2 ± 0.5	2.0 ± 0.9	3.0 ± 1.1	4.5 ± 0.7
P value versus placebo		0.7	0.17	0.07	0.006
BMI (kg/m ²)					
Baseline	32.8 ± 1.6	31.3 ± 1.2	30.8 ± 1.3	32.2 ± 1.6	30.4 ± 1.0
Week 26	32.7 ± 1.9	31.3 ± 1.3	31.6 ± 1.5*	33.2 ± 1.8*	32.0 ± 1.3†
Change	-0.1 ± 0.5	0.1 ± 0.2	0.7 ± 0.3	1.0 ± 0.4	1.0 ± 0.3
P value versus placebo		0.8	0.18	0.11	0.006
HbA _{1c} (%)					
Baseline	8.6 ± 0.5	8.9 ± 0.4	8.0 ± 0.3	8.5 ± 0.5	9.1 ± 0.3
Week 26	9.8 ± 0.8	9.2 ± 0.4	7.9 ± 0.5	7.6 ± 0.5*	7.4 ± 0.3†
Change	1.2 ± 0.5	0.3 ± 0.4	-0.1 ± 0.4	-0.8 ± 0.3	1.8 ± 0.4
P value versus placebo		0.14	0.05	0.003	0.002
FPG (mg/dl)					
Baseline	198 ± 22	231 ± 13	192 ± 13	213 ± 18	230 ± 14
Week 26	218 ± 23	217 ± 18	182 ± 15	167 ± 14*	154 ± 7†
Change	21 ± 25	13 ± 17	10 ± 0.8	-46 ± 19	-77 ± 13
P value versus placebo		0.3	0.2	0.04	0.002
FPI (μU/ml)					
Baseline	18 ± 3	25 ± 5	20 ± 4	20 ± 3	21 ± 3
Week 26	20 ± 4	20 ± 3	17 ± 4	14 ± 2	16 ± 2*
Change	2 ± 2	-5 ± 4	-3 ± 1	-5 ± 3	-5 ± 2
P value versus placebo		0.14	0.07	0.05	0.02
Total cholesterol (mg/dl)					
Baseline	230 ± 2	193 ± 7	205 ± 10	223 ± 14	209 ± 15
Week 26	231 ± 6	198 ± 5	207 ± 11	215 ± 9	214 ± 14
Change	1 ± 14	4 ± 5	3 ± 7	-8 ± 10	5 ± 7
P value versus placebo		0.8	0.9	0.6	0.8
HDL cholesterol (mg/dl)					
Baseline	43 ± 3	40 ± 3	40 ± 2	37 ± 2	41 ± 3
Week 26	46 ± 3	42 ± 3	45 ± 3	42 ± 3†	46 ± 3†
Change	3 ± 2	2 ± 1	5 ± 2	6 ± 1	5 ± 1
P value versus placebo		0.7	0.6	0.2	0.3
LDL cholesterol (mg/dl)					
Baseline	143 ± 14	120 ± 5	123 ± 10	139 ± 12	129 ± 12
Week 26	131 ± 7	119 ± 7	120 ± 9	132 ± 8	133 ± 12
Change	-12 ± 13	-1 ± 6	-3 ± 5	-6 ± 1	5 ± 8
P value versus placebo		0.4	0.5	0.7	0.3
Triglycerides (mg/dl)					
Baseline	248 ± 34	169 ± 7	224 ± 48	256 ± 33	198 ± 24
Week 26	301 ± 56	185 ± 22	205 ± 36	204 ± 39	174 ± 17
Change	53 ± 56	16 ± 17	-19 ± 21	-53 ± 39	-24 ± 22
P value versus placebo		0.3	0.09	0.05	0.08

Data are means ± SEM or n. *P* < 0.05 vs. baseline; †*P* < 0.01 vs. baseline. *P* < 0.01 vs. baseline C, Caucasian; AA, African-American; MA, Mexican-American; A, Asian.

reaching significance in the 45-mg/day group (Table 1). In the 30- and 45-mg/day pioglitazone groups, plasma HDL

cholesterol increased significantly from baseline, and plasma triglyceride concentration tended to decrease compared with

placebo (Table 1). Plasma total and LDL cholesterol did not change significantly from baseline or from the placebo group.

Table 2—Changes in plasma glucose and insulin concentration, insulinogenic index, and hepatic and whole-body ISI during OGTT at baseline and after 26 weeks of pioglitazone treatment

	Placebo	Pioglitazone			
		7.5 mg/day	15 mg/day	30 mg/day	45 mg/day
Mean PG (mg/dl)					
Baseline	322 ± 28	341 ± 16	293 ± 16	342 ± 21	351 ± 19
Week 26	336 ± 35	327 ± 19	290 ± 23	271 ± 19†	258 ± 12*
Change	14 ± 34	-14 ± 19	-3 ± 17	-70 ± 19	-94 ± 21
P value versus placebo		0.5	0.6	0.04	0.01
Mean PI (μU/ml)					
Baseline	43 ± 7	40 ± 5	46 ± 7	38 ± 8	36 ± 6
Week 26	39 ± 9	37 ± 4	42 ± 8	38 ± 8	43 ± 7
Change	-5 ± 6	-3 ± 5	-4 ± 7	0 ± 1	7 ± 4
P value versus placebo		0.9	0.9	0.4	0.095
ΔAUC glucose (mg/dl × min)					
Baseline	14,890 ± 925	13,216 ± 849	12,598 ± 897	15,434 ± 650	14,566 ± 745
Week 26	14,125 ± 1,745	13,152 ± 692	12,100 ± 538	12,545 ± 898†	12,529 ± 687*
Change	-766 ± 1,734	-65 ± 636	-498 ± 893	-2,888 ± 604	-2,037 ± 1,113
P value versus placebo		0.7	0.9	0.3	0.5
ΔAUC insulin (μU/ml × min)					
Baseline	3,064 ± 655	1,788 ± 274	3,172 ± 691	2,137 ± 682	1,833 ± 481
Week 26	2,222 ± 691	1,957 ± 472	3,069 ± 557	2,854 ± 847	3,260 ± 540†
Change	-842 ± 564	169 ± 510	-103 ± 703	717 ± 413	1,427 ± 424
P value versus placebo		0.2	0.4	0.03	0.004
IGI (120 min)					
Baseline	0.22 ± 0.05	0.14 ± 0.02	0.28 ± 0.07	0.15 ± 0.06	0.13 ± 0.03
Week 26	0.21 ± 0.07	0.16 ± 0.04	0.26 ± 0.05	0.27 ± 0.10*	0.27 ± 0.05†
Change	-0.01 ± 0.04	0.02 ± 0.04	-0.02 ± 0.06	0.12 ± 0.05	0.14 ± 0.04
P value versus placebo		0.6	0.9	0.07	0.02
Hepatic ISI					
Baseline	0.15 ± 0.02	0.11 ± 0.02	0.15 ± 0.02	0.13 ± 0.02	0.11 ± 0.02
Week 26	0.13 ± 0.02	0.13 ± 0.03	0.21 ± 0.04	0.21 ± 0.03*	0.24 ± 0.06*
Change	-0.01 ± 0.02	0.02 ± 0.01	0.06 ± 0.03	0.08 ± 0.03	0.13 ± 0.05
P value versus placebo		0.08	0.05	0.02	0.009
Whole-body ISI					
Baseline	1.93 ± 0.29	1.52 ± 0.25	1.92 ± 0.28	1.82 ± 0.26	1.61 ± 0.19
Week 26	2.03 ± 0.39	1.84 ± 0.32	2.34 ± 0.30	2.49 ± 0.29*	2.69 ± 0.57*
Change	0.09 ± 0.19	0.32 ± 0.15	0.42 ± 0.23	0.67 ± 0.25	1.08 ± 0.44
P value versus placebo		0.4	0.3	0.08	0.05

Data are n or means ± SEM. P < 0.05 versus baseline. †P < 0.01 versus baseline. Mean PG, mean plasma glucose concentration during OGTT; Mean PI, mean plasma insulin concentration during OGTT; ΔAUC glucose, (mean PG during OGTT - FPG) × 120 min; ΔAUC insulin, (mean PI during OGTT - fasting PI) × 120 min, IGI (120 min), insulinogenic index = ΔAUC insulin/ΔAUC glucose from 0 to 120 min during OGTT.

OGTT

During the baseline study, there were no statistically significant differences in FPG or FPI concentrations, the incremental area under the plasma glucose curve (ΔAUC glucose) and the incremental area under the plasma insulin curve (ΔAUC insulin) during the OGTT, the insulinogenic index (ΔAUC insulin/ΔAUC glucose) from 0 to 120 min, the hepatic insulin sensitivity index, or the whole-body ISI between the placebo and each pioglitazone group (Table 2). After 26 weeks of treatment with 30 and 45 mg/

day of pioglitazone, the PG concentration during OGTT was significantly reduced from baseline (P < 0.01–0.05) and from placebo (P < 0.01–0.05) (Table 2). In both the 30- and 45-mg/day pioglitazone groups, the ΔAUC glucose was also significantly reduced compared with the pretreatment OGTT (Table 2). After 26 weeks, the incremental plasma insulin responses during the OGTT were similar in the placebo, 7.5-mg pioglitazone, and 15-mg/day pioglitazone groups. However, the ΔAUC insulin increased significantly in the 30-mg/day pioglitazone group (P =

0.03 versus placebo) and the 45-mg/day pioglitazone group (P < 0.01 versus placebo and versus baseline). After 26 weeks of pioglitazone treatment, the insulinogenic index (ΔAUC insulin/ΔAUC glucose from 0 to 120 min) increased significantly in the 30-mg/day pioglitazone group (P < 0.05 versus baseline and P = 0.07 versus placebo) and the 45-mg/day pioglitazone group (P ≤ 0.02 versus placebo and baseline) (Table 2). The hepatic ISI increased significantly versus placebo in the 15-, 30-, and 45-mg/day pioglitazone treatment groups (Table 2).

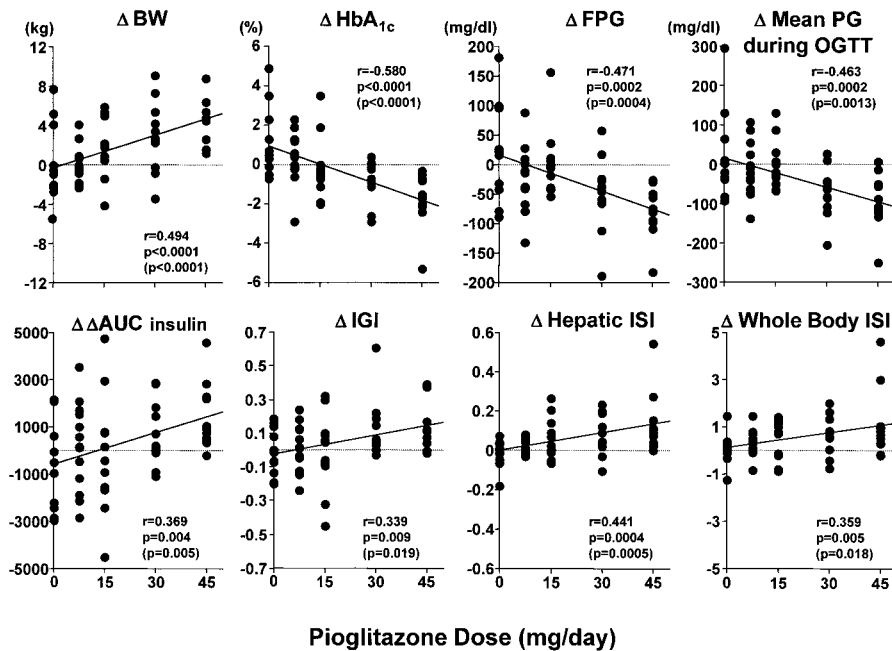


Figure 1—Dose-response relation between pioglitazone and the changes in body weight (BW), HbA_{1c}, FPG, mean PG during the OGTT, the incremental insulin area during the OGTT (Δ AUC insulin), insulinogenic index from 0 to 120 min [IGI (120 min)], hepatic ISI, and whole-body ISI in patients with type 2 diabetes. The Pearson's correlation coefficient and associated P value are shown. The Spearman's correlation coefficient is shown in parentheses.

The whole-body ISI increased in the 30- and 45-mg/day pioglitazone treatment groups compared with baseline and placebo (Table 2).

Correlation analyses

The relationships between pioglitazone dose and change in the selected variables during the 26-week pioglitazone treatment period are shown in Fig. 1. Using Pearson's correlation coefficient, significant positive associations were observed between the pioglitazone dose and increases in body weight, plasma insulin response during OGTT, insulinogenic index, hepatic ISI, and whole-body ISI. Significant negative associations were observed between the pioglitazone dose and decrements in HbA_{1c}, FPG, and mean PG concentration during OGTT. Using Spearman's correlation coefficient, we also examined the association by rank among the five groups (placebo and four pioglitazone groups) and the change in the same selected variables. Highly significant associations between the pioglitazone dose and change in each variable were also observed (Fig. 1; see P values within parentheses).

CONCLUSIONS— This dose-ranging study demonstrates that pioglitazone, given as monotherapy in doses of 30 and 45 mg/day, reduces FPG concentration and glucose excursion during OGTT. The decreases in fasting and post-OGTT PG were associated with decrements in HbA_{1c} of 0.8 and 1.6%, respectively, from baseline and 2.0 and 2.9%, respectively, from placebo. In the diabetic group treated with 15 mg/day of pioglitazone, there was a significant decrease in HbA_{1c} compared with placebo (1.3%, $P < 0.05$) but not compared with baseline (Table 1).

The Δ AUC glucose during OGTT decreased significantly from baseline in the 30- and 45-mg/day pioglitazone groups (Table 1). This beneficial effect of pioglitazone on postprandial hyperglycemia is quite distinct from sulfonylureas (12) and metformin (13), which exert their primary effect on the FPG concentration. Although the meglitinides reduce the postprandial glucose excursion, their effect on FPG is quite modest (14). The improvement in postprandial hyperglycemia after pioglitazone treatment could result from 1) increased insulin secretion, 2) enhanced tissue (peripheral and/or he-

aptic) sensitivity to insulin, or 3) an improvement in the combined effects of hyperglycemia plus hyperinsulinemia to promote glucose metabolism.

The effect of thiazolidinedione treatment on insulin secretion in patients with type 2 diabetes is controversial (1,11,15,16). Some studies have reported a decrease in plasma insulin response to a glucose challenge (1,11), whereas others have failed to observe any significant change (15,16). We found an increase in plasma insulin response during OGTT in diabetic patients treated with 30 and 45 mg/day of pioglitazone (Table 2). An increased or maintained plasma insulin response in the presence of a decreased PG concentration suggests an improvement in β -cell function. Consistent with this, pioglitazone (30- and 45-mg/day dose) increased the insulinogenic index (Δ AUC insulin/ Δ AUC glucose) (Table 2). We believe that the variable effect of thiazolidinediones on the plasma insulin response to a glucose challenge is explained by two opposing effects: 1) a decrease in fasting and postprandial glucose concentrations, leading to reduced glucose toxicity (17); and 2) an increase in insulin sensitivity, leading to reduced insulin secretion (18).

Previous studies with thiazolidinediones in patients with type 2 diabetes concluded that their primary mechanism of action is related to improved insulin sensitivity (1,16,19,20). However, each of these insulin clamp studies used pharmacologic insulin infusion rates (80,120, or 300 $\mu\text{U} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$), which produced supraphysiologic plasma insulin concentrations. In a recent study (8) using more physiologic plasma insulin concentrations, pioglitazone had only a very modest effect to enhance insulin sensitivity in patients with type 2 diabetes. In the present study, 26 weeks of pioglitazone treatment (30 and 45 mg/day) significantly increased the whole-body ISI compared with baseline and compared with placebo (Table 2). This improvement in whole-body ISI was observed with mean plasma insulin concentrations of 36–46 $\mu\text{U}/\text{ml}$ (Table 2). It is important to note that there are significant differences between the OGTT and euglycemic insulin clamp, including the presence (OGTT) or absence (euglycemic insulin clamp) of hyperglycemia and the route of glucose administration (oral ver-

sus intravenous). Moreover, in contrast to the insulin clamp, the whole-body ISI during OGTT is greatly influenced by 1) glucose-mediated glucose uptake and the combined effects of hyperinsulinemia plus hyperglycemia to augment glucose disposal, 2) less effective suppression of hepatic glucose production (21), and 3) splanchnic uptake of 30–40% of the ingested glucose load (21). With regard to the latter, Kawamori et al. (22) have suggested that pioglitazone augments splanchnic glucose uptake after glucose ingestion. These considerations suggest that mechanisms, in addition to improved insulin sensitivity in muscle, contribute to the improvement in whole-body insulin sensitivity during OGTT after pioglitazone therapy.

From the dose-response standpoint, the insulin-sensitizing effect of pioglitazone is clearly evident at doses of 30 and 45 mg/day (Table 2). There was a tendency for whole-body insulin sensitivity to increase at 15 mg/day, but this did not reach statistical significance. Although whole-body insulin sensitivity and dose of pioglitazone were linearly related (Fig. 1), the threshold for a clinically significant insulin-sensitizing effect on glucose homeostasis is ~15 mg/day of pioglitazone.

The product of basal hepatic glucose production (measured with tritiated glucose) and the FPI concentration provides a direct measure of hepatic insulin resistance under postabsorptive conditions, whereas the inverse provides a measure of hepatic insulin sensitivity (9). Because basal hepatic glucose production is closely correlated with FPG concentration (23), the inverse of the product of FPG and insulin concentrations provides an index of hepatic insulin sensitivity (9). The hepatic ISI increased in the 15-, 30-, and 45-mg/day pioglitazone groups ($P < 0.05$ – 0.01 versus placebo). Previous studies examining the effect of troglitazone on basal hepatic glucose production provided apparently contradictory results (1,16,20). In two studies, troglitazone reduced hepatic glucose production (1,20), whereas one study (16) found no effect of troglitazone on basal glucose output by the liver. However, these studies failed to relate the basal rate of hepatic glucose production to the fasting plasma insulin concentration. Because troglitazone treatment was associated with a decrease in FPI, a decreased or even unchanged rate of hepatic glucose production implies an

improvement in hepatic insulin sensitivity.

In type 2 diabetic subjects, weight gain causes worsening of insulin resistance and deterioration of glycemic control. In the present study, pioglitazone treatment (15–45 mg/day) for 26 weeks was associated with mean weight gain of 2.0–4.5 kg, which was significantly and inversely correlated with decreases in HbA_{1c} ($r = -0.527$, $P < 0.0001$), FPG ($r = -0.381$, $P = 0.03$), and mean PG concentration during OGTT ($r = -0.462$, $P < 0.001$). Improved glycemic control, despite weight gain, has been reported with other thiazolidinediones, including rosiglitazone and troglitazone (11,15). Although decreased glucosuria (associated with improved glycemic control) could contribute to the weight gain, this would not be expected to enhance insulin sensitivity. The seemingly paradoxical relationship between weight gain and improved glucose homeostasis/insulin sensitivity most likely is explained by the basic cellular mechanism of action of the thiazolidinediones, which exert their effects through the PPAR γ (3,4). PPAR γ receptors are found primarily in adipose tissue (3), and their activation causes preadipocytes to differentiate into mature small fat cells (3,4). PPAR γ activation also induces key enzymes involved in lipogenesis in these newly formed adipocytes (3,4). Plasma free fatty acids provide the major source of lipid for triglyceride synthesis in these newly formed adipocytes (3). Although PPAR γ receptors are present in both visceral and subcutaneous adipose tissue in humans, thiazolidinediones cause the differentiation of preadipocytes into mature adipocytes only in subcutaneous fat depots (24). This most likely explains the finding that thiazolidinedione-induced weight gain is associated with an increase in subcutaneous fat tissue and a decrease in visceral abdominal fat content (15,25). Because increased visceral fat is associated with insulin resistance (26), a reduction in visceral fat would be expected to lead to an enhancement in insulin sensitivity. Moreover, elevated plasma free fatty acid concentrations/oxidation are common in type 2 diabetic patients and cause insulin resistance in both liver and muscle (27,28). Because thiazolidinedione treatment consistently reduces plasma free fatty acid levels (11,20,29), this may provide another explanation for the improve-

ment in insulin sensitivity despite weight gain.

Dose-response analyses demonstrated significant linear, positive associations between the pioglitazone dose and decrements in HbA_{1c}, FPG, and mean PG concentration during OGTT, as well as between pioglitazone dose and increases in body weight, Δ AUC insulin, insulinogenic index, and hepatic and whole-body ISI (Fig. 1). These significant associations were observed whether or not the placebo group was included in the analysis. Although we cannot determine the maximally effective dose of pioglitazone from the present study, it can be concluded that pioglitazone (at doses ranging from 15 to 45 mg/day) causes a dose-dependent decrease in fasting and postprandial PG concentrations through improvements in hepatic/whole-body insulin sensitivity and in β -cell function in type 2 diabetic patients. For most metabolic parameters, statistically significant changes from baseline and from placebo are evident with pioglitazone doses of 30 and 45 mg/day, with a tendency for the changes to become significant at 15 mg/day. Although some type 2 diabetic patients may respond to a pioglitazone dose of 15 mg/day when given as monotherapy, most will require ≥ 30 mg/day to see clinically significant effects on insulin sensitivity, enhanced β -cell function, and improved glucose homeostasis.

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