The Effect of Pioglitazone on the Liver

Role of adiponectin

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OBJECTIVE — Diabetic hyperglycemia results from insulin resistance of peripheral tissues and glucose overproduction due to increased gluconeogenesis (GNG). Thiazolidinediones (TZDs) improve peripheral insulin sensitivity, but the effect on the liver is less clear. The goal of this study was to examine the effect of TZDs on GNG.

RESEARCH DESIGN AND METHODS — Twenty sulfonylurea-treated type 2 diabetic subjects were randomly assigned (double-blind study) to receive pioglitazone (PIO group; 45 mg/day) or placebo (Plc group) for 4 months to assess endogenous glucose production (EGP) (3-^{3}H-glucose infusion), GNG (D_2O technique), and insulin sensitivity by two-step hyperinsulinemic-euglycemic clamp (240 and 960 pmol/min per m^2).

RESULTS — Fasting plasma glucose (FPG) (10.0 ± 0.8 to 7.7 ± 0.7 mmol/l) and HbA_{1c} (9.0 ± 0.4 to 7.3 ± 0.6%) decreased in the PIO and increased in Plc group ($P < 0.05$ PIO vs. Plc). Insulin sensitivity increased ~40% during high insulin clamp after pioglitazone ($P < 0.01$) and remained unchanged in the Plc group ($P < 0.05$ PIO vs. Plc). EGP did not change, while GNG decreased in the PIO group (9.6 ± 0.7 to 8.7 ± 0.6 $\mu mol \cdot min^{-1} \cdot kg_{ffm}^{-1}$) and increased in the Plc group (8.0 ± 0.5 to 9.6 ± 0.8) ($P < 0.05$ PIO vs. Plc). Change in FPG correlated with change in GNG flux ($r = 0.63$, $P < 0.003$) and in insulin sensitivity ($r = 0.59$, $P < 0.01$). Plasma adiponectin increased after pioglitazone ($P < 0.001$) and correlated with ΔFPG ($r = -0.54$, $P < 0.03$), ΔGNG flux ($r = -0.47$, $P < 0.05$), and Δinsulin sensitivity ($r = 0.65$, $P < 0.005$). Plasma free fatty acids decreased after pioglitazone and correlated with ΔGNG flux ($r = 0.54$, $P < 0.02$). From stepwise regression analysis, the strongest determinant of change in FPG was change in GNG flux.

CONCLUSIONS — Pioglitazone improves FPG, primarily by reducing GNG flux in type 2 diabetic subjects.

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Thiazolidinediones (TZDs) exert their antidiabetes effect by binding to and activating peroxisome proliferator–activated receptor (PPAR)γ (1). PPARγ is expressed predominantly in adipose tissue but also has been found in muscle, liver, pancreas, heart, and spleen, although at much lower levels (2–4). Since PPARγ is mainly expressed in adipose tissue, the effect of TZDs on other organs, such as the liver, is still debated and has not been extensively investigated. TZD treatment has a significant effect on muscle and fat to improve their insulin sensitivity (5,6). In fat, TZDs also stimulate adipocyte differentiation (7), cause fat redistribution (8,9), decrease lipolysis, and augment lipogenesis (10).

While TZDs improve hepatic insulin sensitivity (5,6), basal rates of endogenous glucose production (EGP) have been reported to be both reduced (6) and unchanged (5), although the hepatic insulin resistance index (basal EGP times fasting plasma insulin concentration) consistently declines. TZDs also increase circulating adiponectin levels, an adipocyte-derived cytokine that has been associated with improved peripheral (11) and hepatic (9,12) insulin sensitivity. The effect of TZDs on the liver can be mediated through several pathways. In perfused rat livers, as well as in isolated hepatocytes, TZDs acutely inhibit the rate of glucose release, mainly as a result of reduced gluconeogenesis (GNG) from lactate (13–16). TZDs have been shown in rat liver to inhibit PEPCK, glucose-6-phosphatase, and pyruvate carboxylase, i.e., key enzymes of the GNG pathway (17). Moreover, TZD treatment significantly reduces free fatty acid (FFA) release from adipose tissue (5,6) and decreases visceral fat content (8,18), both of which could lead to the inhibition of GNG.

The goal of the present study was to investigate in type 2 diabetic patients the effect of pioglitazone, a potent PPARγ agonist (19), on the liver by measuring in vivo GNG, glycogenolysis, abdominal fat distribution, and adiponectin levels before and after treatment.

RESEARCH DESIGN AND METHODS

— We studied 20 type 2 diabetic subjects of Mexican-American or non-Hispanic white ethnicity. Characteristics of the study population are shown in Table 1. Only healthy diabetic subjects, as assessed by medical history, physical examination, routine screening blood tests, urinalysis, and electrocardiogram, were studied. Other than sulfonylureas, no subject was taking any medication known to affect glucose/lipid metabolism. Of the subjects treated with pioglitazone, two were on chlorpropamide, four were on glipizide, and four were on glyburide. In the patients treated with placebo, five were on glipizide, one was on chlorpropamide, three were on glyburide, and one

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Abbreviations: EGP, endogenous glucose production; FFA, free fatty acid; FPG, fasting plasma glucose; GNG, gluconeogenesis; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione.

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

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Table 1—*Clinical characteristics and insulin clamp data*

	Placebo		Pioglitazone	
	Pre	Post	Pre	Post
n	10		10	
Ethnicity (Mexican American/non-Hispanic white)	6/4		5/5	
Age (years)	53 ± 4		53 ± 4	
Duration of diabetes (years)	4 ± 1		6 ± 2	
BMI (kg/m^2)	30.2 ± 1.3	30.1 ± 1.3	28.6 ± 1.1	30.0 ± 1.1*
Weight (kg)	83 ± 5	83 ± 5	84 ± 4	88 ± 4*
Fat-free mass (kg)	58 ± 4	58 ± 4	58 ± 2	58 ± 2
Fat mass (kg)	25 ± 3	25 ± 5	26 ± 2	30 ± 3†
Visceral fat (cm^2)	139 ± 25	164 ± 30	150 ± 28	127 ± 31*
Subcutaneous fat (cm^2)	386 ± 34	370 ± 39	252 ± 46	305 ± 56*
A1C (%)	7.8 ± 0.3	8.0 ± 0.4	9.0 ± 0.4	7.3 ± 0.6†
Fasting adiponectin (μg/ml)	7.8 ± 0.7	8.7 ± 1.2	7.6 ± 1.3	17.6 ± 2.5*
Fasting leptin (ng/ml)	13.6 ± 2.8	12.7 ± 2.8	5.5 ± 1.4	5.9 ± 1.0
Fasting glucose (mmol/l)	9.3 ± 0.6	10.6 ± 1.1	10.0 ± 0.8	7.7 ± 0.7†
Fasting insulin (pmol/l)	112 ± 26	103 ± 19	89 ± 17	61 ± 8
Fasting FFAs (μmol/l)	622 ± 50	676 ± 55	610 ± 36	463 ± 33†
Insulin clamp [basal (−30 + 0 min)]				
EGP (μmol · min^{-1} · kg$_{ffm}^{-1}$)	13.9 ± 0.5	14.5 ± 0.9	16.0 ± 0.9	15.6 ± 0.5
Hepatic insulin resistance index (μmol · min^{-1} · kg$_{ffm}^{-1}$ per pmol/l)	1,495 ± 296	1,445 ± 240	1,398 ± 240	947 ± 126*
GNG (%)	57 ± 3	65 ± 3	60 ± 4	56 ± 4*
GNG flux (μmol · min^{-1} · kg$_{ffm}^{-1}$)	8.0 ± 0.5	9.6 ± 0.8	9.6 ± 0.7	8.7 ± 0.6*
Glycogenolytic flux (μmol · min^{-1} · kg$_{ffm}^{-1}$)	5.9 ± 0.4	5.0 ± 0.4	6.5 ± 0.8	6.9 ± 0.7
Glucose clearance (ml · min^{-1} · kg$_{ffm}^{-1}$)	1.8 ± 0.1	1.6 ± 0.1	1.9 ± 0.1	2.4 ± 0.2†
First clamp step (90–120 min)				
EGP (μmol · min^{-1} · kg$_{ffm}^{-1}$)	5.3 ± 0.4	7.0 ± 1.2	6.6 ± 1.1	3.3 ± 0.9†
% GNG	39 ± 5	52 ± 7	40 ± 6	26 ± 4*
GNG flux (μmol · min^{-1} · kg$_{ffm}^{-1}$)	5.9 ± 0.8	6.4 ± 0.9	6.4 ± 0.9	4.8 ± 0.6
Metabolic clearance rate of glucose/insulin (ml · min^{-1} · kg$_{ffm}^{-1}$ per nmol/l)	8.4 ± 1.1	8.1 ± 0.9	11.9 ± 3.5	13.8 ± 2.2
Second clamp step (210–240 min)				
EGP (μmol · min^{-1} · kg$_{ffm}^{-1}$)	2.5 ± 0.8	2.4 ± 0.9	2.6 ± 0.8	1.9 ± 0.5
Metabolic clearance rate of glucose/insulin (ml · min^{-1} · kg$_{ffm}^{-1}$ per nmol/l)	4.2 ± 0.4	3.7 ± 0.4	5.0 ± 1.4	7.2 ± 1.6†

Data are means ± SE. Placebo-adjusted differences: *$P < 0.05$–0.01 vs. placebo; †$P < 0.01$ or less vs. placebo.

was on glimepiride. The sulfonylurea dose was maintained constant throughout the study.

Body weight was stable for at least 3 months before study, and no subject was participating in a heavy exercise program before or during the study. Subjects were asked to consume a weight-maintaining diet containing 50% carbohydrate, 30% fat, and 20% protein for 3 days before each study. Studies were carried out at the General Clinical Research Center of the University of Texas Health Science Center at San Antonio following an overnight fast. The study protocol was approved by the institutional review board of the University of Texas Health Science Center at San Antonio, and informed written consent was obtained from each patient before participation. The data on muscle glucose uptake in type 2 diabetic patients are part of a larger dataset, which previously has been published (5) and are used here to specifically investigate the relationship between EGP and GNG. Only patients (20 of 23) who received $[^2H]_2O$ were here analyzed.

The design of the study has been described elsewhere (5). Briefly, all subjects underwent a 75-g oral glucose tolerance test (OGTT) with measurement of lean body mass and fat mass using an intravenous bolus of $[^3H_2]O$. On a separate day, a euglycemic-hyperinsulinemic clamp study was performed with [3-^{3}H] glucose infusion (to measure EGP and peripheral tissue sensitivity to insulin) and $[^2H]_2O$ ingestion (to measure the separate contributions of GNG and glycogenolysis to EGP). Following completion of these studies, patients were randomized to receive pioglitazone (PIO group; 45 mg/day) or placebo (Plc group) with breakfast every day for 16 weeks. Patients returned to the Clinical Research Center every 2 weeks for follow-up visits, and, during the last week of treatment, all metabolic studies were repeated. In a subgroup of subjects (six patients treated with pioglitazone and six with placebo), quantitation of abdominal subcutaneous and visceral fat area at L4-L5 was performed using magnetic resonance imaging.

OGTT

Subjects were admitted to the Clinical Research Center at 0800 following a 12-h overnight fast and received an OGTT (75 g) with measurement of plasma glucose, FFAs, and insulin concentrations every

15 min from −30 to 0 and from 0 to 120 min. At time zero, a 100-μCi intravenous bolus of [$^3H_2$]O was given and plasma [$^3H_2$]O radioactivity was determined at 90, 105, and 120 min. Lean and fat body mass were calculated as previously described (5).

Euglycemic-hyperinsulinemic clamp

Subjects were admitted to the Clinical Research Center at 0700, after a ~13-h overnight fast, and a spontaneously voided urine sample was obtained. Subjects were asked not to change their habitual diet regimen, to eat the last meal between 1800 and 1900, and not to eat or drink anything after the last meal. At 2200 on the evening before the study, all subjects drank [$^2H_2$]O (5 g/kg fat free mass; Isotech). A fasting baseline blood sample was taken in the morning on the day before the study for the determination of [$^2H_2$]O enrichment. On arrival at the Clinical Research Center, a polyethylene cannula was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted retrogradely into an ipsilateral wrist vein on the dorsum of the hand for blood sampling, and the hand was kept in a heated box at 65°C. A prime (25 μCi × fasting plasma glucose[FPG]/5)-continuous (0.25 μCi/min) infusion of 3-[^{3}H]glucose (DuPont-NEN, Boston, MA) was initiated and continued until the end of the study. During the last 30 min of the basal equilibration period (150–180 min), blood samples were taken at 5- to 10-min intervals for the determination of plasma glucose and insulin concentrations and [3-^{3}H] glucose specific activity. After the basal equilibration period, insulin was administered as a prime-continuous infusion at the rate of 240 pmol/min per m^2 for 90 min and then at a rate of 960 pmol/min per m^2 for another 90 min, as previously described (5). The plasma glucose concentration was measured every 5 min after the start of the insulin infusion, and a variable infusion of 20% glucose was adjusted based on the negative feedback principle to maintain the plasma glucose level at ~5 mmol/l with a coefficient of variation <5%. Blood samples were collected every 15 min from 0 to 60 and from 90 to 150 min and every 5–10 min from 60 to 90 and from 150 to 180 min for the determination of plasma glucose and insulin concentrations and [3-^{3}H] glucose specific activity. Samples for the determination of plasma [^{2}H] enrichment were taken before starting the [3-^{3}H] glucose infusion, at the end of the basal tracer equilibration period, and after 90 min of insulin infusion.

Analytical methods

Plasma glucose concentration was determined by the glucose oxidase method (Beckman II Glucose Analyzer; Beckman, Fullerton, CA). Plasma insulin concentration was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). HbA_{1c} (A1C) was measured by affinity chromatography (Biochemical Methodology, Drower 4350; Isolab, Akron, OH). Plasma FFA concentration was measured spectrophotometrically (Wako Chemicals, Neuss, Germany). [3-^{3}H] glucose specific activity was measured on barium hydroxide/zinc sulfate deproteinized plasma samples (Somogyi procedure). Plasma adiponectin and leptin concentrations were measured by radioimmunoassay (Linco Research, St. Charles, MO).

The pattern of ^{2}H incorporation into plasma glucose after [$^2H_2$]O ingestion was determined according to the method developed by Landau and colleagues (20,21), as recently modified. Briefly, the fraction of glucose produced via GNG from all precursors can be quantified from the ratio of ^{2}H enrichment of carbon 5 (C5) to that of water. ^{2}H enrichment at C5 was obtained by converting glucose to xylose by the removal of carbon in position 6, after purification by high-performance liquid chromatography. The C5 group was cleaved by oxidation with periodic acid and the formaldehyde was collected by distillation and incubated with ammonia to form a molecule of hexamethylenetetramine. Enrichment of hexamethylenetetramine obtained from C5 was determined by gas chromatography–mass spectrometry by monitoring peaks of mass 140 and 141. Precision and accuracy of C5 have been reported previously (22).

[^{2}H] enrichment in the body water pool was monitored by reacting a sample of urine with calcium carbide (CaC_2) to obtain acetylene (C_2H_2), and the enrichment of C_2H_2 was then determined by gas chromatography–mass spectrometry by monitoring peaks of mass 26 and 27 (23). All samples were run through gas chromatography–mass spectrometry processing in duplicate or triplicate.

Data analysis

Total body water was calculated from the mean ^{3}H-water radioactivity at 90, 105, and 120 min after the bolus of ^3H_2O. Plasma tritiated water specific activity was calculated assuming that plasma water represents 93% of plasma volume. Fat-free mass equals body water divided by 0.73 (24). Subcutaneous and visceral fat areas were quantitated by magnetic resonance imaging at the L4-L5 level, as previously described (25). All glucose fluxes were expressed per kilogram of fat-free mass, since this normalization has been shown to correct for differences due to sex, obesity, and age (26). During the last 30 min of the basal tracer equilibration period, both the plasma glucose concentration and [^{3}H] glucose specific activity were stable in all subjects. Therefore, total EGP was calculated as the ratio of the [^{3}H] glucose infusion rate to the plasma [^{3}H] glucose specific activity (mean of five determinations). During the euglycemic clamp, the total glucose rate of appearance (R_a) was calculated using Steele's equation. EGP was then obtained as the difference between R_a and the exogenous glucose infusion rate. The tracer-determined rate of glucose disappearance (R_d) provided a measure of insulin-mediated total body glucose disposal.

Since the fasting plasma insulin concentration is a strong inhibitory stimulus for EGP (27), the basal hepatic insulin resistance index (in units of $\mu mol \cdot min^{-1} \cdot kg_{ffm}^{-1}$ per pmol/l) was calculated as the product of fasting EGP and fasting plasma insulin concentration. Experimental validation for the use of this index has been published (28,29). Peripheral insulin sensitivity was calculated as the mean R_d during each clamp step divided by the mean plasma glucose concentration (i.e., the glucose metabolic clearance rate) divided by the mean plasma insulin concentration (in units of $ml \cdot min^{-1} \cdot kg_{ffm}^{-1}$ per nmol/l).

The percent contribution of GNG to plasma glucose was calculated as the ratio of the enrichments in C5/[$^2H_2$]O (21). Gluconeogenic flux was calculated by multiplying percent GNG by EGP. The glycogenolytic flux was obtained as the difference between EGP and the gluconeogenic flux. During the two-step euglycemic insulin clamp, [$^2H_2$]O enrichment is not altered by the infused fluid (30). In contrast, C5 enrichment is diluted by the exogenous glucose. Therefore, the C5-to-[$^2H_2$]O ratio gives the contribution of GNG to the total (endogenous plus exogenous) concentration of glucose in the plasma. By applying the standard precursor–product relationship, gluconeogenic flux was calculated by

multiplying the C5-to-[$^2H_2$]O ratio by the total glucose R_a at any time point during the insulin clamp (30).

Statistical analysis

Data are given as the means ± SE. For each measured variable, the effect of treatment was tested by regressing the change of the variable (>16 weeks) against the baseline value (as a continuous variable) and the group (pioglitazone versus placebo) and calculating the interaction term between the two independent variables. Placebo-adjusted differences (means ± SE) were calculated with the use of contrasts. The contribution of multiple factors to the decrease in FPG was assessed by multivariate and stepwise regression analysis; for this analysis, variables with skewed distribution were log transformed. A P value of ≤0.05 was considered significant.

RESULTS

Body weight, A1C, fasting glucose/insulin/FFAs

At baseline, the PIO and Plc groups were well matched for age, duration of diabetes, and BMI (Table 1). After 16 weeks of treatment, BMI and fat mass increased in the PIO group ($P < 0.05$) and remained unchanged in the Plc group (Table 1). FPG and A1C decreased significantly in the PIO group and increased slightly in the Plc group (both $P < 0.01$ PIO vs. Plc). The fasting plasma FFA concentration decreased in the PIO group and rose in the Plc group ($P < 0.01$). The fasting plasma insulin concentration declined slightly in the PIO group, but the change fell short of statistical significance (Table 1). The mean plasma glucose concentration during the OGTT declined from 16.5 ± 0.8 to 12.7 ± 0.9 mmol/l in the PIO group ($P < 0.003$) and rose slightly in the Plc group (14.0 ± 0.9 to 15.6 ± 1.2) (P = NS). The mean plasma insulin concentration during the OGTT did not change significantly either in the PIO group (196 ± 44 to 195 ± 57 pmol/l) or in the Plc group (357 ± 128 vs. 296 ± 89 pmol/l).

EGP and GNG: fasting state

At baseline, fractional GNG from all precursors was similar in the Plc and PIO groups (Table 1). After 16 weeks of treatment, both the fractional GNG (placebo-adjusted change = −4 ± 4%, $P = 0.05$) and the total GNG flux (placebo-adjusted change = −1.5 ± 0.7 μmol · min^{-1} · kg$_{ffm}^{-1}$, $P = 0.02$) decreased significantly with pioglitazone, while no significant change occurred in glycogenolytic flux. Total EGP remained unchanged. The hepatic insulin resistance index declined markedly by 35% in the PIO group (−451 ± 196 μmol · min^{-1} · kg$_{ffm}^{-1}$ per pmol/l, $P < 0.05$ vs. baseline and vs. Plc). The change in GNG flux was positively correlated with the changes in FPG and FFA concentrations (Fig. 1). Glucose clearance during the fasting state was significantly enhanced by pioglitazone (placebo-adjusted change = +0.5 ± 0.2 ml · min^{-1} · kg$_{ffm}^{-1}$, $P < 0.005$).

In the subgroup of subjects in whom abdominal fat distribution was measured by magnetic resonance imaging, pioglitazone treatment was associated with increased subcutaneous abdominal fat ($P < 0.01$) and reduced visceral fat ($P < 0.01$). In the whole dataset, a direct correlation was found between the change in GNG flux and the change in the visceral-to-subcutaneous adipose tissue ratio (Fig. 1).

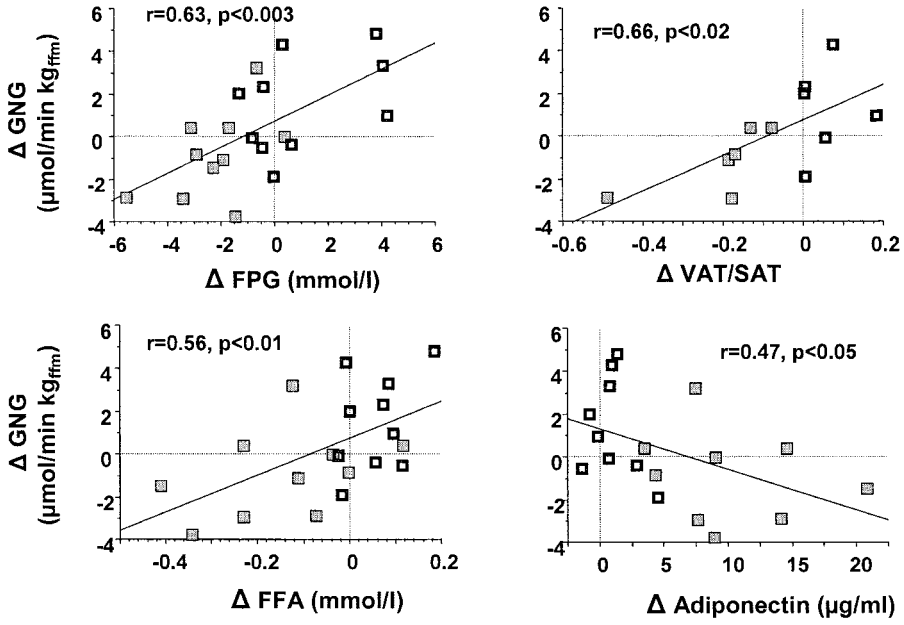

Figure 1—*Relationship between change in gluconeogenic flux and changes in FPG/adiponectin/FFA concentrations and change in visceral-to-subcutaneous adipose ratio in patients treated with pioglitazone (■) and placebo (□).*

Euglycemic clamp

During the first insulin clamp step, steady-state plasma glucose (5.5–6.4 mmol/l), insulin (381–432 pmol/l), and FFA (170–224 umol/l) concentrations were similar pre- and posttreatment with pioglitazone and placebo. During the second insulin clamp step, the steady-state plasma glucose (5.0–5.1 mmol/l), insulin (1,909–2,091 pmol/l), and FFAs (141–168 umol/l) also were similar in all clamp studies.

In the PIO group, the lower insulin infusion rate was associated with a 78% suppression of EGP and a 30% inhibition of fractional GNG; GNG flux was responsible for virtually all of EGP under these conditions (Table 1). Pioglitazone treatment was associated with a small improvement in peripheral tissue insulin sensitivity (metabolic clearance rate/insulin, placebo-adjusted change = +1.9 ± 2.5 ml · min^{-1} · kg$_{ffm}^{-1}$ per nmol/l, P = NS). At the higher insulin infusion rate, EGP was similarly suppressed (by ~90%) in both groups, while peripheral insulin sensitivity was significantly improved by pioglitazone (placebo-adjusted change = +2.2 ± 0.5 ml · min^{-1} · kg$_{ffm}^{-1}$ per nmol/l, $P < 0.005$).

Adipocytokines

Pioglitazone treatment significantly increased plasma adiponectin concentration (Table 1), and the change was inversely correlated with changes in FPG concentration ($r = -0.54$, $P < 0.03$), residual EGP during 240 pmol/min per m^2 clamp step ($r = -0.74$, $P < 0.0005$), and gluconeogenic flux ($r = -0.47$, $P < 0.05$) (Fig. 1) and positively correlated (r = 0.60, $P < 0.009$) with change in total body glucose disposal. No change in plasma leptin concentration was observed following pioglitazone treatment.

Correlations

In the whole dataset, the decrement in FPG concentration was associated with decreases in visceral fat mass ($r = 0.88$, $P < 0.0005$), GNG ($r = 0.63$, $P < 0.003$), and fasting plasma FFAs ($r = 0.51$, $P < 0.03$) and with increases in glucose disposal rate during high-dose insulin clamp ($r = -0.77$, $P < 0.0002$) and plasma adiponectin concentration ($r = -0.54$, $P < 0.03$). Using multiple and stepwise regression models, we examined which were the main determinants (changes in basal FFA and adiponectin levels, GNG flux, glycogenolytic flux, and insulin sensitivity) of change in FPG. Adiponectin was included in the model, since it is known to inhibit EGP (31). In both multiple regression and stepwise models, the strongest determinant of the change in FPG was the change in GNG flux (partial $r = 0.54$, $P < 0.05$).

CONCLUSIONS — Diabetic hyperglycemia results from impaired insulin action on skeletal muscle and excessive endogenous glucose release due to increased GNG (30). TZDs improve both peripheral and hepatic insulin sensitivity (5,6). Studies (16,31) in rodents indicate that TZDs decrease gluconeogenesis. At the cellular liver, TZDs inhibit expression of PEPCK, pyruvate carboxylase, and glucose-6-phosphatase, key genes involved in regulation of GNG and total hepatic glucose production (17). TZDs also increase expression of glucokinase (17), providing an explanation for their ability to enhance splanchnic glucose uptake (9). TZDs also inhibit lipolysis, thereby reducing plasma glycerol and FFA levels (6,18) (Table 1). By improving oxidative glucose metabolism in peripheral tissues, TZDs also would be expected to reduce Cori cycle activity and decrease lactate delivery to the liver. An insulin-sensitizing effect of adiponectin on the liver also has been demonstrated in rodents (32), and we previously have shown a strong correlation between the increase in plasma adiponectin concentration and the reduction in endogenous (primarily hepatic) glucose production following TZD treatment (9).

In a recent publication, we have shown that rosiglitazone administration to drug-naïve/drug-withdrawn type 2 diabetic patients decreases basal gluconeogenic flux in parallel with the reduction in FPG concentration (33). Both pioglitazone and rosiglitazone are PPARγ agonists and belong to the TZD class of oral hypoglycemic agents. As a class, PPARγ agonists turn on and off many of the same genes (34). However, each PPARγ agonist has its own individual effect to stimulate/inhibit the expression of specific genes. This is particularly relevant to pioglitazone and rosiglitazone, which have very different effects on lipid metabolism (35,36). In the present study, we have evaluated the effect of pioglitazone treatment for 4 months in sulfonylurea-treated type 2 diabetic patients and examined the relationship between changes in GNG flux and plasma adiponectin levels.

Compared with placebo, pioglitazone significantly reduced the fasting rate of GNG, and the decrease was correlated with the decline in FPG concentration. Following pioglitazone, total basal hepatic glucose production did not decline significantly because the decrease in GNG was partially offset by a small increase in glycogenolysis, i.e., hepatic autoregulation. Pioglitazone markedly enhanced hepatic insulin sensitivity, as manifested by a 35% decrease in the hepatic insulin resistance index (Table 1). During low-dose insulin clamp, EGP was suppressed by ~78% as the result of a 30% fall in GNG flux and complete inhibition of glycogenolysis (30,37). Pioglitazone enhanced insulin-mediated glucose clearance by 16% during the low-dose insulin clamp step (P = NS) and by 44% during the high-dose insulin clamp step ($P < 0.01$). Thus, under euglycemic-hyperinsulinemic conditions, pioglitazone improved insulin sensitivity of glucose uptake by peripheral tissues, while under fasting conditions, it improved hepatic insulin sensitivity and reduced gluconeogenic flux. The pioglitazone-induced decrement in GNG flux was the strongest determinant of the drug's antihyperglycemic effect. A recent study (12) demonstrated improved hepatic insulin sensitivity within 21 days after institution of TZD treatment in type 2 diabetic patients, at a time when body weight, fat distribution, and peripheral insulin sensitivity were unchanged. Taken together with the current results, these findings indicate that liver is an important primary target tissue of TZD action. However, it should be noted that the FPG concentration fell significantly without a significant decrease in hepatic glucose output. This decline in fasting glucose was accounted for by the increase in basal glucose clearance, indicating enhanced tissue sensitivity to the ambient fasting plasma insulin concentration.

In the present study, the total plasma adiponectin concentration increased significantly in the PIO group and the change in plasma adiponectin correlated strongly with the change in both GNG and FPG concentration, as well as with the increase in peripheral (muscle) and hepatic insulin sensitivity. A small rise in adiponectin levels has been shown to reduce hepatic glucose production in mice by decreasing glucose flux through glucose-6-phosphatase, in association with reduced activity of glucose-6 phosphatase and PEPCK (17). In the study by Tonelli et al. (12), total plasma adiponectin concentration did not change during TZD treatment. Rather, the improvement in hepatic insulin sensitivity was strongly associated with the change in high–molecular weight form of adiponectin. The present results, in combination with those of Tonelli et al. (12) suggest that the decrease in GNG may be mediated, in part, via the increase in adiponectin, although further work is necessary to define which species of circulating adiponectin is responsible for the adipocytokine's effect on the liver. We speculate that adiponectin may function as the insulin-sensitizing coordinator that mediates cross-talk between adipose tissue, muscle, and liver.

It is well established that chronic TZD treatment induces adipocyte differentiation (7) and fat redistribution (decreased visceral and increased subcutaneous adipose content) (8,9), inhibits lipolysis (38), and augments lipogenesis (10). In our patients, pioglitazone treatment caused modest increases in total fat mass and subcutaneous abdominal fat content, which paralleled the improvements in glycemia and decrease in GNG. Although an increase in fat mass typically is associated with TZD treatment (8,39), increased total body fat content is not a requirement for the glucose-lowering action of TZDs (40). Rather, weight gain appears to be a distinct pharmacological effect that parallels and predicts the improvements in insulin sensitivity and glycemic control (5,8,9,18). In very hyperglycemic patients, some portion of the weight gain is explained by reduced glucosuria (41). Improved insulin sensitivity in adipose tissue (38,42), resulting in decreased release of FFAs, particularly from visceral fat depots (43), and increased FFA uptake by subcutaneous adipocytes (44) lead to a decline in plasma FFA concentration, which plays a central role in the insulin-sensitizing effects of the TZDs (rev. in 18). Excess visceral fat has been

associated with increased GNG flux and poor glycemic control in type 2 diabetic patients (45). Recently, it has been shown that release of FFA and gluconeogenic substrates into the portal circulation is increased with visceral fat accumulation (46). According to the "portal theory" (47), this should lead to increased GNG flux. The results of the present study demonstrate that the reduction in GNG parallels the reduction in circulating FFA. One could, therefore, speculate that TZD-induced change in abdominal fat distribution, with reduced visceral and increased subcutaneous fat content, is an important mechanism via which TZDs improve hepatic glucose metabolism. Although not measured in the present studies, we (9,47,48) and others (49) have shown that TZDs also reduce hepatic fat content and that the reduction in hepatic fat content is associated closely with improved hepatic insulin sensitivity.

In summary, our results demonstrate that the liver is an important target for TZD action, and inhibition of GNG flux is a key determinant of pioglitazone's antihyperglycemic effect. This finding in sulfonylurea-treated type 2 diabetic patients is consistent with results obtained in drug-naïve type 2 diabetic patients treated with rosiglitazone (33). The efficacy of PPARγ agonists can be attributed to *1*) improved hepatic insulin sensitivity during the postabsortive state, resulting in decreased hepatic glucose production; *2*) improved muscle insulin sensitivity under conditions of hyperinsulinemia, resulting in increased tissue glucose uptake; *3*) improved adipose tissue insulin sensitivity resulting in decreased FFA release; and *4*) increased circulating levels of adiponectin.

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