Comparison of the Effects of Pioglitazone and Metformin on Hepatic and Extra-Hepatic Insulin Action in People With Type 2 Diabetes

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OBJECTIVE—To determine mechanisms by which pioglitazone and metformin effect hepatic and extra-hepatic insulin action.

RESEARCH DESIGN AND METHODS—Thirty-one subjects with type 2 diabetes were randomly assigned to pioglitazone (45 mg) or metformin (2,000 mg) for 4 months.

RESULTS—Glucose was clamped before and after therapy at ~5 mmol/l, insulin raised to ~180 pmol/l, C-peptide suppressed with somatostatin, glucagon replaced at ~75 pg/ml, and glycerol maintained at ~200 mmol/l to ensure comparable and equal portal concentrations on all occasions. Insulin-induced stimulation of glucose disappearance did not differ before and after treatment with either pioglitazone (23 ± 3 vs. 24 ± 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) or metformin ($22 \pm 2$ vs. 24 ± 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). In contrast, pioglitazone enhanced ($P < 0.01$) insulin-induced suppression of both glucose production (6.0 ± 1.0 vs. 0.2 ± 1.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and gluconeogenesis ($n = 11$; 4.5 ± 0.9 vs. 0.8 ± 1.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Metformin did not alter either suppression of glucose production (5.8 ± 1.0 vs. 5.0 ± 0.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) or gluconeogenesis ($n = 9$; 3.7 ± 0.8 vs. 2.6 ± 0.7 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). Insulin-induced suppression of free fatty acids was greater ($P < 0.05$) after treatment with pioglitazone (0.14 ± 0.03 vs. 0.06 ± 0.01 mmol/l) but unchanged with metformin (0.12 ± 0.03 vs. 0.15 ± 0.07 mmol/l).

CONCLUSIONS—Thus, relative to metformin, pioglitazone improves hepatic insulin action in people with type 2 diabetes, partly by enhancing insulin-induced suppression of gluconeogenesis. On the other hand, both drugs have comparable effects on insulin-induced stimulation of glucose uptake. ***Diabetes*** **57: 24–31, 2008**

Thiazolidinediones and metformin are extensively used to treat people with type 2 diabetes. Both are considered to be insulin "sensitizers" (1–5). However, the mechanism of action of these drugs remains an area of active investigation. Although it is commonly stated that thiazolidinediones lower glucose concentration primarily by increasing glucose uptake and metformin by decreasing glucose production (1–5), the data supporting these statements are scarce and often contradictory. In vitro and animal studies have identified multiple potential targets for these drugs (6–12). Both increase insulin signaling and muscle as well as adipocyte glucose uptake (6–8,11). Both have been shown to modulate the activity of hepatic enzymes, particularly those involved in the gluconeogenic pathway (9,10,12–16). In contrast, the results have not always been consistent in humans (rev. in 17). The effects of metformin have been particularly variable with some (18–21) but not all (22–25) showing an improvement in insulin action. However, because any therapy that lowers glucose concentration (e.g., treatment with insulin or sulfonylurea) improves insulin action (26–28), it has been difficult to distinguish between a specific effect of the thiazolidinedione or metformin and that due to the associated reduction in glucotoxicity in placebo-controlled trials.

The relative ability of these agents to modulate hepatic and extra-hepatic insulin action is uncertain because most studies have compared the effects of a thiazolidinedione or metformin with that observed during treatment with placebo (14,19,21,26–31) or before treatment (13,15,16,18) rather than to one another. To our knowledge, the exception is the recent study by Tiikkainen et al. (32). In this carefully designed clinical trial, previously untreated subjects with type 2 diabetes were randomly assigned to receive either rosiglitazone or metformin for 16 weeks. Hepatic insulin action, when estimated by multiplying fasting insulin concentration times fasting endogenous glucose production, comparably improved in both groups after treatment. On the other hand, when hepatic insulin action was assessed during a hyperinsulinemic-euglycemic clamp, suppression of glucose production was greater after treatment with metformin than treatment with rosiglitazone. However, the insulin concentrations were higher during the clamp with metformin than with rosiglitazone, thereby confounding interpretation of the data.

In an effort to gain greater insight regarding the mechanism of action of these commonly used therapeutic agents, hepatic and extra-hepatic insulin action was measured in people with type 2 diabetes using a hyperinsulinemic-euglycemic clamp before and after 4 months of

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B.R.L. is deceased.

HMW, high molecular weight.

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TABLE 1
Subject characteristics

	Pioglitazone		Metformin	
	Pretreatment	Post-treatment	Pretreatment	Post-treatment
Subjects	7F/9M	—	8F/7M	—
Age (years)	56 ± 3	57 ± 3	60 ± 2	60 ± 2
Weight (kg)	93.5 ± 3.9	97.6 ± 4.5*	93.5 ± 4.4	94.1 ± 4.3
BMI (kg/m^2)	32.3 ± 1.5	33.8 ± 1.7	31.7 ± 1.3	32.0 ± 1.3
Lean body mass (kg)	52.9 ± 2.4	53.4 ± 2.2	52.4 ± 3.4	53.0 ± 3.3
Body fat (%)	37.9 ± 2.1	40.0 ± 2.1*	37.9 ± 2.3	38.7 ± 2.4
Visceral fat (cm^2)	212 ± 21	225 ± 19	213 ± 24	220 ± 25
Fasting glucose (mg/dl)	157 ± 10	140 ± 13	148 ± 12	146 ± 10
A1C	6.5 ± 0.2	6.2 ± 0.2	6.3 ± 0.2	7.0 ± 0.3*

*$P < 0.05$ pretreatment vs. post-treatment.

treatment with either pioglitazone or metformin. We specifically sought to test the hypothesis that in the presence of physiological insulin concentrations, suppression of endogenous glucose production is greater after treatment with a thiazolidinediones than after treatment with metformin. To accurately assess hepatic insulin action, insulin was raised to concentrations that in the absence of treatment with a sensitizer are known to result in submaximal suppression of endogenous glucose production (32). C-peptide was suppressed with somatostatin, glucagon replaced at ~75 pg/ml, and glycerol maintained at ~200 mmol/l to insure comparable and equal portal concentrations on all occasions. Gluconeogenesis and glycogenolysis were measured using the deuterated water method. We report that, whereas pioglitazone and metformin have comparable effects on insulin-induced stimulation of glucose uptake, pioglitazone improved hepatic insulin action at least in part by enhancing insulin-induced suppression of gluconeogenesis.

RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 31 subjects with type 2 diabetes gave informed written consent to participate in the study. All subjects were in good health and at a stable weight. None regularly engaged in vigorous physical exercise. After completion of the pretreatment study, subjects were randomly assigned (double-blind, double-placebo controlled) to receive either pioglitazone (45 mg daily) or metformin (1,000 mg twice daily) for 4 months. Of the 16 subjects in the pioglitazone group, 12 had been previously treated with metformin alone, 1 with sulfonylurea alone, 2 with a combination of metformin and sulfonylurea, and 1 with diet alone. Of the 15 subjects in the metformin group, 12 had been previously treated with metformin alone, 1 with sulfonylurea alone, and 2 with combination of metformin and sulfonylurea. Oral hypoglycemic medications were discontinued at least 10 days before the pretreatment study visit. After randomization, pioglitazone was started at 30 mg daily for the 1st week and then increased to 45-mg dose at the end of the 1st week. Metformin was started at 500 mg once daily and then increased in increments of 500 mg/week to a maximum dose of 2,000 mg within 4 weeks. Subjects continued to receive the pioglitazone or metformin during the post-treatment study, i.e., study drugs were not discontinued during the evening before and morning of study, and each subject took their regular and study medications as prescribed. Subjects on stable doses of thyroxine, estrogen replacement therapy, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, and metabolically neutral antihypertensive medication (low-dose thiazide, calcium channel blockers, or losartan) continued these medicines during the study.

All subjects were instructed to follow a weight maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein for at least 3 days before the day of study. Subjects were admitted to the Mayo Clinic Clinical Research Unit at 1700 h on the evening before the study. A standard 10 cal/kg meal (55% carbohydrate, 30% fat, and 15% protein) was eaten between 1730 and 1800 h. After sampling blood for baseline enrichment, 1.67 ^2H_2O/kg weight of body water was given in three divided doses at 1800, 2000, and 2200 h. Small sips of water (containing ^2H_2O) were permitted on request. After the meal, an 18-gauge catheter was inserted into a forearm vein and an infusion of insulin was started (100 units regular human insulin in 1 l 0.9% saline containing 5 ml 25% human albumin). The insulin infusion rate was adjusted to maintain glucose concentrations at ~5 mmol/l during the night (33).

An infusion of glycerol was started at 0600 h in amounts equal to that given as part of a separate protocol in which subjects also were infused with Intralipid. A primed (fasting glucose in millimoles per liter divided by 5.5 mmol/l × 12 μCi) continuous (0.12 μCi/min) infusion of [3-^{3}H]glucose (Perkin Elmer, Boston, MA) at 0700 h and infusions of insulin (0.6 mU · kg^{-1} · min^{-1}), somatostatin (60 ng · kg^{-1} · min^{-1}), growth hormone (3 ng · kg^{-1} · min^{-1}), and glucagon (0.65 ng · kg^{-1} · min^{-1}) were started at 1000 h (time 0 min). An infusion of [9,11,12,12-$^2H_4$]cortisol also was started at 0600 h as part of a separate protocol. Beginning at 0930 h, glucose was infused in amounts sufficient to maintain plasma glucose concentrations at ~5 mmol/l. All infused glucose contained [3-^{3}H]glucose to minimize the change in plasma glucose specific activity. In addition, the rate of the "basal" [3-^{3}H]glucose infusion was varied to mimic the anticipated changes in endogenous glucose production.

Analytical techniques. Blood samples were collected in prechilled syringes and dispensed into prechilled tubes. Samples for free fatty acids were collected in tubes containing 50 μl of Paraoxon (diethyl-*p*-nitrophenylphophate) (Sigma Chemicals, St Louis, MO) diluted to 0.04% in diethyl ether to prevent ex vivo lipolysis. Samples were placed in ice, centrifuged at 4°C, and separated. All other samples were stored at −20°C until analysis. Plasma glucose was measured by a glucose oxidase method using a glucose analyzer (YSI, Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence method with the Access Ultrasensitive Immunoenzymatic assay system (Beckman, Chaska, MN). C-peptide and glucagon concentrations were assayed by radioimmunoassay (Linco Research, St. Louis, MO). [3-^{3}H]glucose specific activity was measured by liquid scintillation counting as previously described. Plasma glycerol and free fatty acid concentrations were measured by a modified microfluorometric enzymatic method (34) using the Cobas, MIRA analyzer (Roche). Enrichment of deuterium on the second and fifth carbons of plasma glucose was measured as previously described (35). Body composition (including fat-free mass, total fat mass, and visceral fat mass) was measured using dual energy X-ray absorptiometry (DPX scanner, Lunar, Madison, WI) combined with a computerized tomograph scan at the level of L2/L3 as previously described (36). Velocity sedimentation/gel filtration chromatography was used for separation of adiponectin complexes using a human adiponectin radioimmunoassay kit (Linco) in eight subjects in the pioglitazone group and eight subjects in the metformin group.

Calculations. Rates are expressed in the figures and text as micromoles per kilogram lean body mass per minute. Basal and clamp responses were assessed by taking the mean of the values present from −30 to 0 min and from 270 to 300 min, respectively. Glucose appearance and disappearance were calculated using the steady-state equations of Steele et al. (37). Endogenous glucose production during the clamp was calculated by subtracting the exogenous glucose infusion rate from the total glucose appearance rate. The rate of gluconeogenesis was calculated by multiplying the plasma ratio of C5 and C2 glucose enrichments times endogenous glucose production (38). Glycogenolysis was calculated by subtracting the rate of gluconeogenesis from endogenous glucose production. Rates of gluconeogenesis and glycogenolysis could be measured in 20 of the 31 subjects ($n = 11$ in the pioglitazone group and 9 in the metformin group). Rates could not be measured in the other subjects because of high baseline C5 enrichment.

Statistical analysis. Data in the text and figures are expressed as means ± SE. Student's paired t test was used to determine whether values were different before and after treatment. A P value of <0.05 was considered as statistically significant.

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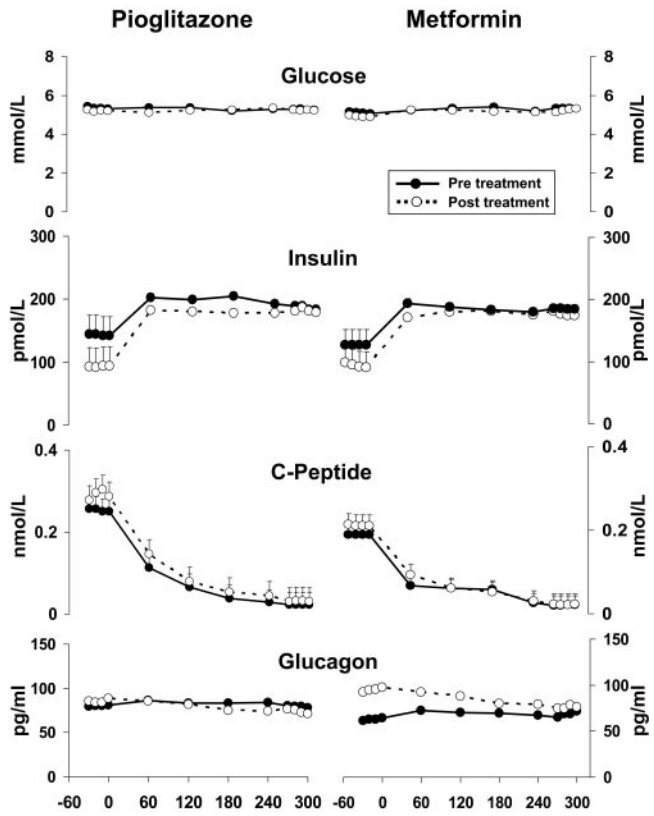

FIG. 1. Plasma glucose, insulin, C-peptide, and glucagon concentrations observed before and during infusion of somatostatin in diabetic subjects both before and after treatment with pioglitazone and metformin. Somatostatin and replacement infusions of glucagon and growth hormone were started at time 0.

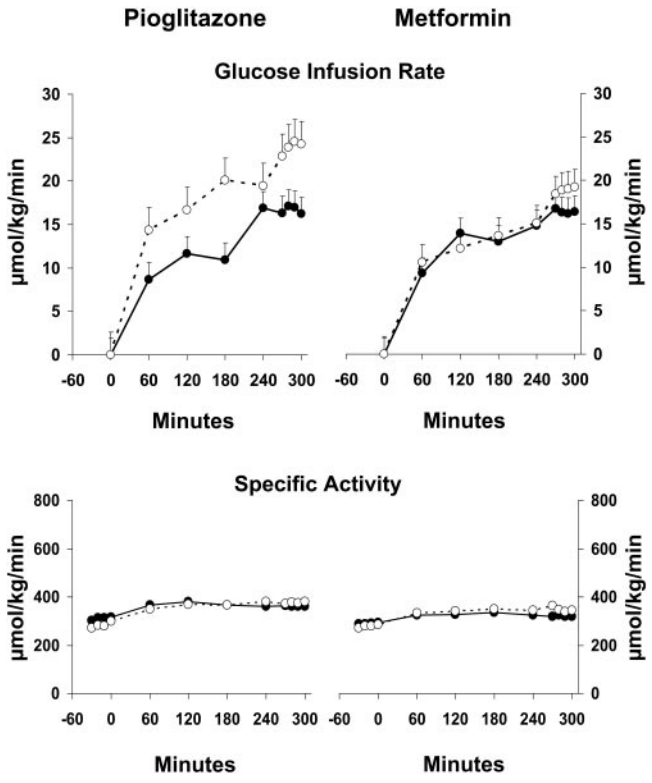

FIG. 2. The intravenous glucose infusion rate required to maintain target glucose concentrations (*top*) and plasma [3-^{3}H]glucose specific activity (*bottom*) observed in the diabetic subjects both before (○) and after (●) treatment with pioglitazone and metformin during final 40 min of a baseline period (basal) and a euglycemic-hyperinsulinemic (clamp) period. Somatostatin, insulin, and replacement infusions of glucagon and growth hormone were started at time 0.

RESULTS

Patient characteristics. Patient characteristics are provide in Table 1. Age, sex, BMI, lean body mass, percent body fat, and visceral fat did not differ between groups before treatment. Body weight and percent body fat were slightly higher ($P < 0.05$) with pioglitazone but remained unchanged with metformin. A1C did not differ before therapy and did not change during treatment with pioglitazone. However, A1C increased ($P < 0.05$) slightly during treatment with metformin.

Glucose, insulin, C-peptide, and glucagon concentrations. Glucose concentrations before and during the final 30 min of the clamp did not differ before or during treatment in either the pioglitazone or metformin groups (Fig. 1).

The insulin infusion rates required to maintain euglycemia during the final hour before the clamp were slightly but not significantly lower after treatment with either metformin (1.7 ± 0.3 vs. 1.2 ± 0.3 units/h) or pioglitazone (1.9 ± 0.3 vs. 1.2 ± 0.3 units/h). Although fasting insulin concentrations after the overnight exogenous insulin infusion also tended to decrease after treatment with either pioglitazone (143 ± 27 vs. 94 ± 21 pmol/l) or metformin (136 ± 22 vs. 97 ± 18 pmol/l), neither change was statistically significant. Plasma insulin concentrations during the final 30 min of the clamp did not differ before and during treatment with either pioglitazone (185 ± 12 vs. 181 ± 9 pmol/l) or metformin (185 ± 10 vs. 178 ± 8 pmol/l).

C-peptide concentrations after the overnight exogenous insulin infusion did not differ during treatment with either pioglitazone (0.25 ± 0.03 vs. 0.29 ± 0.03 nmol/l) or metformin (0.18 ± 0.03 vs. 0.21 ± 0.03 nmol/l). Somatostatin resulted in comparable and near-complete suppression of C-peptide secretion on all occasions.

Plasma glucagon concentrations after the overnight exogenous insulin infusion did not differ before and during treatment with pioglitazone (80 ± 4 vs. 86 ± 6 pg/ml) but were higher ($P < 0.01$) during treatment with metformin (63 ± 4 vs. 95 ± 9 pg/ml). On the other hand, plasma glucagon concentrations during the final 30 min of the clamp did not differ before and during treatment with either pioglitazone (79 ± 5 vs. 74 ± 3 pg/ml) or metformin (68 ± 3 vs. 76 ± 4 pg/ml).

Glucose infusion rate required to maintain euglycemia, glucose specific activity. Compared with pretreatment, the glucose infusion rate required to maintain euglycemia increased ($P < 0.01$) during treatment with pioglitazone (16.7 ± 3.2 vs. 24.1 ± 3.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) but did not differ during treatment with metformin (16.4 ± 3.2 vs. 18.9 ± 3.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (Fig. 2). Glucose specific activity remained constant before and during the clamp both before and after treatment with either pioglitazone or metformin, thereby permitting accurate measurements of glucose turnover.

Endogenous glucose production and glucose disappearance. Endogenous glucose production before the clamp did not differ before and during treatment with either pioglitazone (15.8 ± 0.6 vs. 16.1 ± 0.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) or metformin (17.2 ± 0.7 vs. 17.6 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) (Fig. 3). Suppression of endogenous glucose production during the final 30 min of the clamp was

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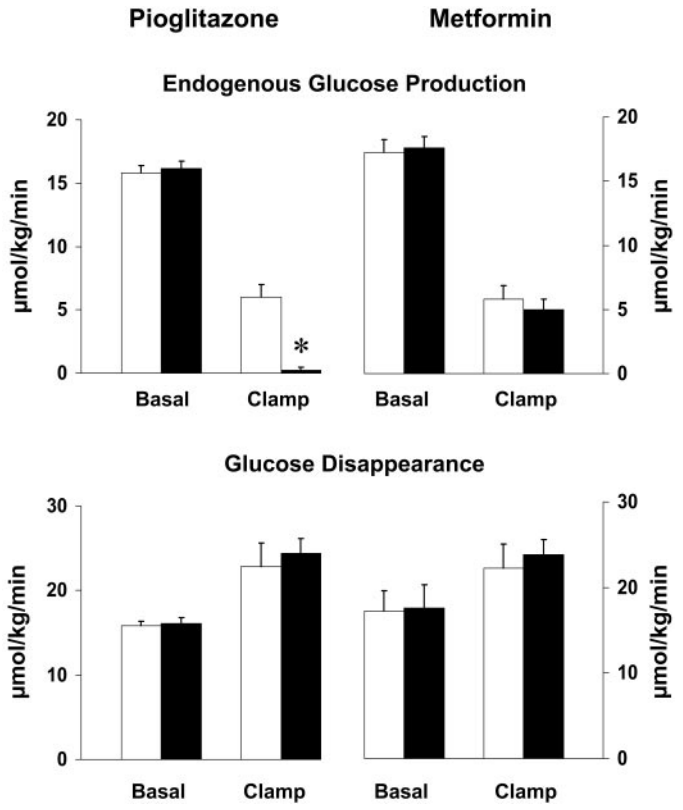

FIG. 3. Rates of endogenous glucose production (*top*) and glucose disappearance (*bottom*) observed in the diabetic subjects both before (□) and after (■) treatment with pioglitazone (*left*) and metformin (*right*) during final 30 min of a baseline period (basal) and a euglycemic-hyperinsulinemic (clamp) period. **P* < 0.001 vs. pretreatment.

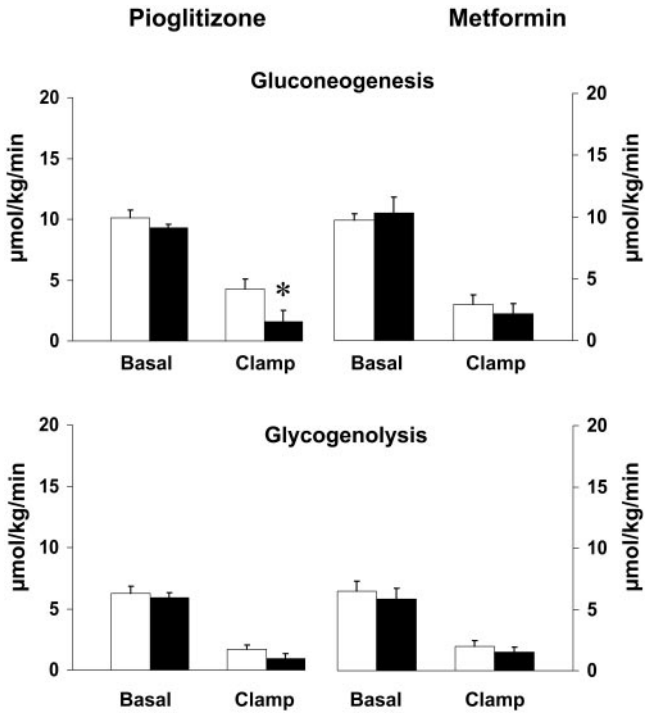

FIG. 4. Rates of gluconeogenesis (*top*) and glycogenolysis (*bottom*) observed in the diabetic subjects both before (□) and after (■) treatment with pioglitazone and metformin during final 30 min of a baseline period (basal) and a euglycemic-hyperinsulinemic (clamp) period. **P* < 0.001 vs. pretreatment.

greater ($P < 0.001$) during treatment with pioglitazone (6.0 ± 1.0 vs. 0.2 ± 1.6 μmol · kg^{-1} · min^{-1}) but did not differ during treatment with metformin (5.8 ± 1.0 vs. 5.0 ± 0.8 μmol · kg^{-1} · min^{-1}).

Glucose disappearance before the clamp did not differ before and during treatment with either pioglitazone (15.8 ± 0.6 vs. 16.1 ± 0.6 μmol · kg^{-1} · min^{-1}) or metformin (17.2 ± 0.7 vs. 17.6 ± 1.1 μmol · kg^{-1} · min^{-1}). Glucose disappearance during the final 30 min of the clamp also did not differ before and during treatment with either pioglitazone (22.8 ± 2.8 vs. 24.4 ± 1.8 μmol · kg^{-1} · min^{-1}) or metformin (22.2 ± 2.5 vs. 23.8 ± 2.8 μmol · kg^{-1} · min^{-1}).

Gluconeogenesis and glycogenolysis. Gluconeogenesis before the clamp did not differ before and during treatment with either pioglitazone (10.6 ± 0.5 vs. 11.1 ± 0.7 μmol · kg^{-1} · min^{-1}) or metformin (9.8 ± 0.7 vs. 10.3 ± 0.9 μmol · kg^{-1} · min^{-1}) (Fig. 4). On the other hand, gluconeogenesis during the final 30 min of the clamp was lower ($P < 0.01$) during than before treatment with pioglitazone (4.5 ± 0.9 vs. 0.8 ± 1.2 μmol · kg^{-1} · min^{-1}) but did not differ during treatment with metformin (3.7 ± 0.8 vs. 2.6 ± 0.7 μmol · kg^{-1} · min^{-1}). Glycogenolysis did not differ before the clamp before and during treatment with pioglitazone (6.3 ± 0.5 vs. 6.3 ± 0.4 μmol · kg^{-1} · min^{-1}) or metformin (7.6 ± 0.7 vs. 7.0 ± 0.7 μmol · kg^{-1} · min^{-1}). On the other hand, suppression of glycogenolysis was greater during the final 30 min of the clamp ($P < 0.01$) after treatment with pioglitazone (2.0 ± 0.4 vs. −0.3 ± 0.9 μmol · kg^{-1} · min^{-1}) but did not differ after treatment with metformin (2.2 ± 0.5 vs. 1.5 ± 0.4 μmol · kg^{-1} · min^{-1}).

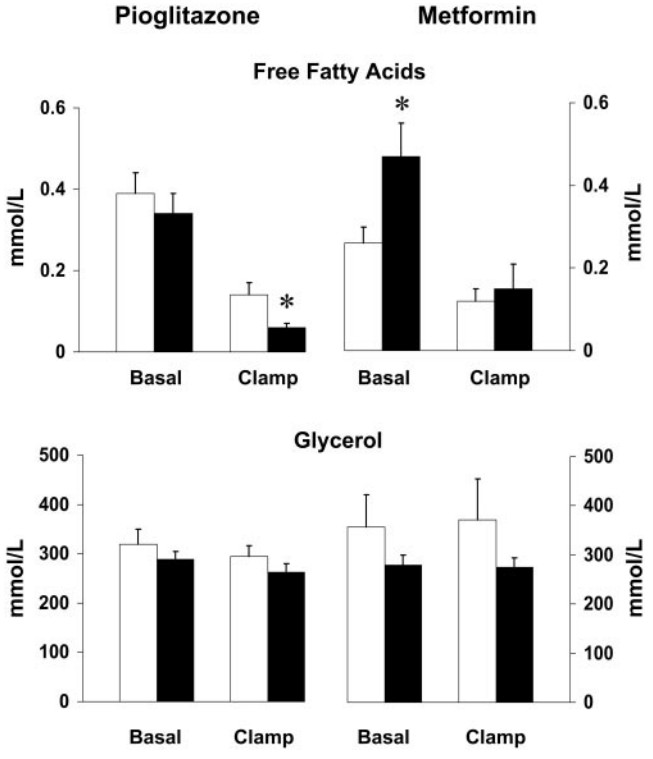

FIG. 5. Free fatty acid concentrations observed in the diabetic subjects both before (□) and after (■) treatment with pioglitazone and metformin during final 30 min of a baseline period (basal) and a euglycemic-hyperinsulinemic (clamp) period. **P* < 0.01 vs. pretreatment.

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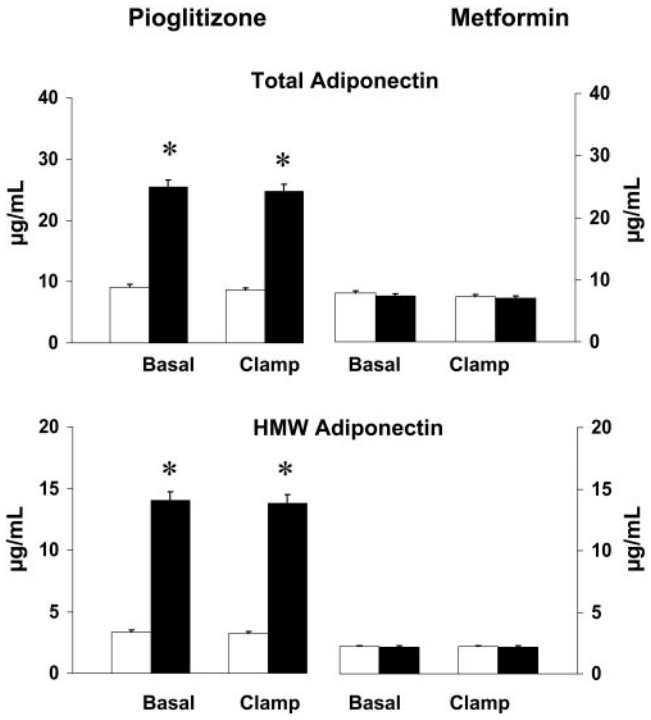

FIG. 6. Total and HMW adiponectin concentrations observed in the diabetic subjects both before (□) and after (■) treatment with pioglitazone and metformin during final 30 min of a baseline period (basal) and a euglycemic-hyperinsulinemic (clamp) period. *P < 0.01 vs. pretreatment.

Free fatty acid and glycerol concentrations. Plasma free fatty acid concentrations before the clamp did not differ before and during treatment with pioglitazone (0.39 ± 0.05 vs. 0.34 ± 0.05 mmol/l) (Fig. 5). On the other hand, treatment with pioglitazone resulted in greater ($P <$ 0.05) suppression of plasma free fatty acid concentrations during the final 30 min of the clamp (0.14 ± 0.03 vs. 0.06 ± 0.01 mmol/l). In contrast, plasma free fatty acids during treatment with metformin were higher ($P < 0.05$) before the clamp (0.26 ± 0.04 vs. 0.47 ± 0.08 mmol/l) but did not differ during the final 30 min of the clamp (0.12 ± 0.03 vs. 0.15 ± 0.07 mmol/l).

Plasma glycerol concentrations did not differ before or during the final 30 min of the clamp before or during treatment with either pioglitazone or metformin.

Total and HMW plasma adiponectin concentrations Treatment with pioglitazone resulted in an marked increase ($P < 0.01$) in both total and high molecular weight (HMW) plasma adiponectin concentrations (Fig. 6). In contrast, neither total nor HMW plasma adiponectin concentrations changed after treatment with metformin.

DISCUSSION

Thiazolidinediones and metformin both are considered insulin "sensitizers" (1–5). The present data indicate that when compared with one another, pioglitazone and metformin have equivalent effects on insulin-induced stimulation of glucose uptake assessed at insulin concentrations that are commonly present under the conditions of daily living. On the other hand, relative to metformin, pioglitazone increases insulin-induced suppression of endogenous glucose production at least in part by enhancing suppression of gluconeogenesis.

Multiple studies have shown that thiazolidinediones improve hepatic insulin action relative to placebo (6, 16,30,31). The present studies establish that thiazolidinediones also improve hepatic insulin action relative to metformin. Insulin-induced suppression of glucose production was greater after 4 months of treatment with pioglitazone but did not differ after 4 months of treatment with metformin. Glucose production was assessed under conditions designed to optimize comparison between therapies. Consistent with previous reports (39–41), treatment with metformin increased fasting glucagon concentrations. However, this does not explain the difference in hepatic insulin sensitivity after treatment with pioglitazone or metformin because endogenous glucagon secretion was inhibited during the clamps with somatostatin. Insulin was infused at rates anticipated to result in submaximal suppression of glucose production so that differences in hepatic response could be evaluated. This design resulted in peripheral (and presumably portal) insulin concentrations during the clamps that were comparable before and during treatment in both groups. Furthermore, the gluconeogenic precursor glycerol, infused as part of a separate protocol evaluating the effects of elevated free fatty acids on glucose metabolism, was maintained at comparable concentrations during treatment with pioglitazone or metformin despite differences in plasma free fatty acid concentrations. Consistent with previous studies, treatment with pioglitazone resulted in both an increase in plasma adiponectin concentration and an improvement in hepatic insulin action (16,42). In contrast, treatment with metformin did not alter either plasma adiponectin concentration (32,43) or hepatic insulin action. These data further support the premise that thiazolidendiones improve insulin action at least in part by increasing adiponectin concentrations (16).

The observation in the present study that hepatic insulin action was enhanced after treatment with pioglitazone but not metformin differs from the report by Tiikkainen et al. (32) that glucose production was lower and percent suppression greater after treatment with metformin but not after treatment with rosiglitazone. Although it is possible that rosiglitazone and pioglitazone have different effects on hepatic insulin action, we doubt that this is the case. More likely, the apparent discordance between the conclusions of the two studies is due to the fact that in the study of Tiikkainen et al. (32), *1*) insulin concentrations were significantly lower on the rosiglitazone than metformin study days, thereby accounting for lesser suppression of glucose production; *2*) glucose concentrations were clamped at hyperglycemic levels (~8 mmol/l), making it difficult to distinguish effects of these agents on glucose effectiveness from those due to changes in insulin action; and *3*) endogenous insulin secretion was not inhibited, leaving open the possibility that differences in suppression of glucose production after treatment were due to differences in portal insulin concentrations rather than differences in hepatic insulin action. Taken together, the current data indicate that when measured under the appropriate conditions (i.e., glucose concentrations clamped at euglycemic levels with portal insulin and glucagon concentrations matched), pioglitazone enhances insulin-induced suppression of glucose production, whereas metformin does not.

Glucose production equals the sum of glycogenolysis and gluconeogenesis. Previous studies have shown that thiazolidinediones and metformin inhibit multiple enzymes in the gluconeogenic pathway and, when compared with

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placebo, lower gluconeogenesis in humans (9,10,12–15,42). In the present studies, insulin-induced suppression of gluconeogenesis and glycogenolysis improved after treatment with pioglitazone but did not change after treatment with metformin. The enhanced suppression of glucose production was accompanied by enhanced insulin-induced suppression of plasma free fatty acid concentration. Because plasma free fatty acids modulate rates of both gluconeogenesis and glycogenolysis, it is possible that pioglitazone improved hepatic insulin sensitivity by enhancing suppression of lipolysis and/or increasing plasma free fatty acid clearance (44–46). However, association or lack thereof does not prove causality. Therefore, future studies will be required to distinguish between the direct effects of thiazolidinediones on hepatic insulin action from those due to their effect in fat metabolism.

Of note, plasma free acid concentrations before the clamp were higher after than before metformin treatment. On the other hand, free fatty acid concentrations suppressed to comparable concentrations during the clamp on both occasions. We have no explanation for the higher basal free fatty acid concentrations after treatment with metformin. However, the higher free fatty acid concentrations may explain, at least in part, why basal rates of glucose production after treatment with metformin did not differ from those present before treatment despite the fact that plasma insulin concentrations tended to be lower. In any case, the current data emphasize the need for further study of the effects of metformin on free fatty acid metabolism in people with type 2 diabetes.

Insulin-induced stimulation of glucose uptake did not differ before and during treatment in either the pioglitazone or metformin groups. This indicates that under the present experimental conditions (i.e., low physiological insulin concentrations), both agents had a comparable effect on extra-hepatic insulin action. Although effects of metformin on insulin action have been inconsistent (18–21), at first glance, the present data may appear at variance with previous reports that thiazolidinediones increase insulin-stimulated glucose uptake (16,29,30). However, whereas an increase in glucose uptake after treatment with a thiazolidinediones almost always has been observed when assessed in the presence of very high insulin concentrations, enhanced uptake was not observed in the same studies when assessed at lower insulin concentrations that still were substantially higher than those used in the present experiments (16,29,30). The one exception was the study of Tiikkainen et al. (32) in which an improvement in insulin-induced stimulation of glucose uptake after treatment with rosiglitazone was detected at insulin concentrations similar to those used in the present experiments (32). However, in those experiments, A1C fell during treatment with rosiglitazone, so reduced glucotoxicity may have contributed to the improvement in insulin action. Taken together, these data suggest that in the presence of low physiological insulin concentrations, the effects of pioglitazone on hepatic insulin action is likely as important, if not more important, than the effects of pioglitazone on extra-hepatic insulin action in the regulation of glucose metabolism.

The present study has certain limitations. Twenty-four of the 31 subjects were being treated with metformin before study, reflecting the standard of practice in our community. Although all glucose-lowering agents were discontinued at least 10 days before the pretreatment study, it could be argued that in these subjects, the design in effect was continuation of their current therapy with metformin versus treatment with pioglitazone. However, this does not detract from the observation that treatment with pioglitazone resulted in an improvement in hepatic insulin action, whereas treatment with metformin did not. Furthermore, the conclusions were the same when the 24 individuals who had been treated with metformin alone before study were analyzed as a separate subset. A1C increased slightly in the metformin group but remained unchanged in the pioglitazone group. Plasma free fatty acids also increased. Therefore it is possible that the deterioration in glycemic control and the associated increase in free fatty acid concentrations blunted the effects of metformin on insulin action. On the other hand, because metformin is not known to have a direct effect on insulin secretion, these changes presumably occurred because insulin action was lower during treatment with metformin than with pioglitazone consistent with the results observed during the clamps.

Subjects in the metformin groups were treated with a maximum of 2,000 mg/day. It is possible that higher doses of metformin would have improved hepatic insulin action. However, we doubt whether this would be the case because the efficacy of metformin appears to plateau at 2,000 mg/day (47). The maximum dose of pioglitazone was reached within 1 week, whereas the maximum dose of metformin was not reached until 1 month. We believe that it is unlikely that the difference in time to maximum dose affected the conclusions because subjects were on stable doses of both drugs for 3 months before study. Insulin was infused during the night to ensure that glucose concentrations were comparable in all groups on all study days, thereby avoiding the confounding effects of "glucotoxicity" due to differences in glycemic control (48). We only compared metformin with pioglitazone. We do not know if the results would have been the same if we used a different thiazolidinedione (e.g., rosiglitazone) because previous studies have suggested that the effects of these agents on blood lipids differ (49). On the other hand, because when compared with placebo the effects of pioglitazone and rosiglitazone on insulin action are the same (30,31), we believe the current data are representative of the response to the thiazolidinedione class of drugs rather than specific for pioglitazone.

In summary, in the presence of low physiological insulin concentrations, insulin-induced stimulation of glucose uptake is comparable during treatment with pioglitazone or metformin. In contrast, insulin-induced suppression of glucose production is greater during treatment with pioglitazone than during treatment with metformin, at least in part, due to enhanced suppression of gluconeogenesis. Free fatty acids also were lower during the clamp after treatment with pioglitazone but not after treatment with metformin, implying that pioglitazone enhances suppression of lipolysis and/or increases FFA clearance. Thus, the common belief that pioglitazone improves glycemic control in people with type 2 diabetes primarily by increasing glucose uptake whereas metformin does so by suppressing glucose production needs to be reexamined.

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