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Effect of rosiglitazone on glucose and non-esterified fatty acid metabolism in Type II diabetic patients

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Abstract

Aims/hypothesis. We aimed to examine the mechanisms by which rosiglitazone improves glycaemic control in Type II (non-insulin-dependent) diabetic patients.

Methods. Altogether 29 diet-treated diabetic patients were assigned at random to rosiglitazone, 8 mg/day ($n = 15$), or placebo ($n = 14$) for 12 weeks. Patients received 75 g OGTT and two-step euglycaemic insulin (40 and 160 mU/m^{2}min) clamp with 3-^{3}H-glucose, ^{14}C-palmitate and indirect calorimetry.

Results. After 12 weeks, rosiglitazone reduced fasting plasma glucose (195 ± 11 to 150 ± 7 mg/dl, $p < 0.01$), mean plasma glucose (PG) during OGTT (293 ± 12 to 236 ± 9 mg/dl, $p < 0.01$), and HbA_{1c} (8.7 ± 0.4 to 7.4 ± 0.3 %, $p < 0.01$) without changes in plasma insulin concentration. Basal endogenous glucose production (EGP) declined (3.3 ± 0.1 to 2.9 ± 0.1 mg/kg FFM · min, $p < 0.05$) and whole body glucose metabolic clearance rate increased after rosiglitazone (first clamp step: 2.8 ± 0.2 to 3.5 ± 0.2 ml/kg FFM · min, $p < 0.01$; second clamp step: 6.7 ± 0.6 to 9.2 ± 0.8, $p < 0.05$) despite increased body weight (86 ± 4 to 90 ± 4 kg, $p < 0.01$) and fat mass (33 ± 3 to 37 ± 3 kg, $p < 0.01$). Fasting plasma non-esterified fatty acid (NEFA) (735 ± 52 to 579 ± 49 μEq/l, $p < 0.01$), mean plasma NEFA during OGTT (561 ± 33 to 424 ± 35, $p < 0.01$), and basal NEFA turnover (18.3 ± 1.5 to 15.5 ± 1.2 μEq/kg FM · min, $p < 0.05$) decreased after rosiglitazone. Changes in EPG and mean plasma glucose (PG) during OGTT correlated with changes in basal EGP ($r = 0.54$; $r = 0.58$), first EGP ($r = 0.36$; $r = 0.41$), first MCR ($r = –0.66$; $r = –0.68$), second MCR ($r = –0.49$; $r = –0.54$), fasting plasma NEFA ($r = 0.53$; $r = 0.49$), and NEFA during OGTT ($r = 0.66$; $r = 0.66$).

Conclusion/interpretation. Rosiglitazone increases hepatic and peripheral (muscle) tissue insulin sensitivity and reduces NEFA turnover despite increased total body fat mass. These results suggest that the beneficial effects of rosiglitazone on glycaemic control are mediated, in part, by the drug's effect on NEFA metabolism. [Diabetologia (2001) 44: 2210–2219]

Keywords Type II diabetes mellitus, rosiglitazone, glucose and non-esterified fatty acid metabolism, insulin sensitivity.

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Abbreviations: PG, Plasma glucose; EGP, endogenous glucose production; MCR, whole body glucose metabolic clearance rate; FFM, fat free mass; FM, fat mass; *PPARγ*, peroxisome proliferator activated receptor gamma; FPG, fasting plasma glucose; Ra, rate of endogenous glucose appearance; TGD, total glucose disposal; SSPI, steady state plasma insulin; GOX, glucose oxidation rate; NOGD, non-oxidative glucose disposal rate

The thiazolidinediones represent a new class of insulin sensitizing agents for the treatment of Type II (non-insulin-dependent) diabetes mellitus. The original drug in this class, troglitazone, has been shown to ameliorate insulin resistance and improve glycaemic control and dyslipidaemia in Type II diabetic patients [1–6]. However, all previous studies which employed the insulin clamp technique to examine the effect of troglitazone on insulin sensitivity employed pharmacologic insulin infusion rates (80–300 mU/m^2 min) that produced supraphysiologic plasma insulin con-

centrations [1–4]. No study has examined the effect of thiazolidinedione on insulin-mediated glucose disposal in Type II diabetic patients at physiologic plasma insulin concentrations.

Thiazolidinediones act by binding to a specific nuclear receptor, peroxisome proliferator activated receptor gamma (PPARγ) [7,8], and their binding affinity to PPARγ closely parallels their in vivo hypoglycaemic potency [9, 10)]. PPARγ receptors are found primarily in adipocytes and their concentration in muscle, the primary tissue responsible for the disposal of an infused glucose load, is low [7, 11]. Consistent with their tissue distribution, PPARγ activation causes pre-adipocytes to differentiate into mature fat cells, and causes the induction of key enzymes involved in lipogenesis [7, 12, 13]. In agreement with these observations, clinical studies have shown that thiazolidinedione therapy in Type II diabetic patients is associated with weight gain. However, despite the weight gain, glycaemic control improves [6, 14–19], and the increase in body weight is positively related to the reduction in HbA_{1C} [16]. These results suggest that improved glucose homeostasis following thiazolidinedione treatment could be related to alterations in lipid metabolism or fat topography or both. Clinical studies have shown that thiazolidinediones causes a 20–30 % reduction in fasting plasma non-esterified fatty acid (NEFA) concentration, which is paralleled by a 20–30 % reduction in fasting plasma glucose concentration and HbA_{1C} in Type II diabetic patients [1, 2, 5, 6, 15–19]. These observations suggest that the primary effect of the thiazolidinediones is to reduce the plasma NEFA concentration by inhibiting NEFA release from adipose tissue [7, 20], leading to improved insulin sensitivity and glycaemic control in Type II diabetic individuals. No previous study has examined the effect of any thiazolidinedione on NEFA metabolism in humans and related observed changes in NEFA turnover to changes in insulin sensitivity and glycaemic control.

In this study, we evaluated the effect of rosiglitazone on glucose tolerance, insulin secretion, peripheral and hepatic sensitivity to insulin, plasma lipid concentrations, and NEFA turnover in Type II diabetic patients. We assessed the action of rosiglitazone or any other thiazolidinedione on hepatic and peripheral insulin sensitivity and NEFA metabolism under basal conditions and in response to a physiologic increment in plasma insulin concentration.

Subjects and methods

Subjects. Twenty-nine Type II diabetic patients participated in the study. Entry criteria included an age of 30–70 years, BMI of less than 38 kg/m^2, and fasting plasma glucose concentration (FPG) between 140–260 mg/dl. Patients previously treated with insulin or another thiazolidinedione were excluded. Patients were in good general health without evidence of cardiac, hepatic, renal or other chronic diseases, as determined by history, examination, and routine blood chemistries. Patients were not taking any medications known to affect glucose metabolism. None were performing any excessive physical activity, and body weight was stable for at least 3 months before the study. All patients gave signed voluntary, informed consent before participation. The protocol was approved by Institutional Review Board of the University of Texas Health Science Center at San Antonio.

Study design. The study had a double blind, placebo controlled, parallel design. In 24 subjects who were taking a sulphonylurea, the medication was discontinued and then all 29 subjects participated in a 6-week single blind placebo run in period. During the run-in placebo period, the FPG was measured weekly. Only patients whose plasma glucose concentration varied by less 5 % during the last 3 weeks of the run in period were allocated at random to rosiglitazone or placebo. Patients met with a dietitian at the start of run-in period and were instructed to consume a weight maintaining diet (50 % carbohydrate, 30 % fat, 20 % protein). During the week before randomization, patients received, firstly, a 75 OGTT; secondly, management of lean body and fat mass with ^3H_2O; thirdly, a two-step euglycaemic insulin clamp with 3-^{3}H-glucose, 1-^{14}C-palmitate, and indirect calorimetry to examine hepatic and peripheral tissue sensitivity to insulin, NEFA turnover and respiratory gas exchange, respectively. The bolus injection of ^3H_2O was done on the same day as the OGTT. All studies were carried out at 0800 h after a 10–12 h overnight fast. Following completion of these studies, patients were allocated at random to receive rosiglitazone, 8 mg/day, or placebo with breakfast for 12 weeks. Patients returned to the Clinical Research Center at 0800 h every 2 weeks for a measurement of their FPG and body weight, and to check compliance. Fasting plasma lipids (total/LDL/HDL cholesterol and triglyceride) and HbA_{1c} were measured monthly. During the last week of the double blind period, the OGTT, euglycaemic insulin clamp, and body fat measurements were repeated.

OGTT. Plasma glucose, NEFA, insulin and C-peptide concentrations were measured at –30, –15 and 0 minutes. At time zero, patients ingested 75 g of glucose and plasma glucose, NEFA, insulin and C-peptide concentrations were measured at 15-min intervals for 2 h. At time zero, a 100 μCi bolus of ^3H_2O was given and plasma tritiated water radioactivity was measured at 90, 105, 120 min to calculate lean body mass and fat mass [21].

Euglycaemic insulin clamp. Insulin sensitivity was assessed with a two-step euglycaemic insulin clamp [22]. At 0800 h (-180 min), a prime (25 μCi x FPG/100)-continuous (0.25 μCi/min) infusion of ^{3}H-3-glucose was started using an antecubital vein catheter. A second catheter was placed retrogradely into a vein on the dorsum of the hand, which was placed in a heated box (60 °C). Baseline arterialized venous blood samples for determination of plasma ^{3}H-3-glucose radioactivity and plasma glucose and insulin concentrations were drawn at –30, –20, –10, –5, and 0 min. At time zero, a prime-continuous infusion of human regular insulin (Novolin, Novo Nordisk, Princeton, N.J. USA) was started at a rate of 40 mU $\cdot$ min^{-1} $\cdot$ m^{-2} for 120 min. At 120 min, the insulin space was reprimed and the insulin infusion rate was increased to 160 mU $\cdot$ min^{-1} $\cdot$ m^{-2} for 120 min. After the start of the insulin infusion, the plasma glucose concentration was allowed to drop to 90–100 mg/dl, and maintained at that concentration by adjusting an infusion of 20 % dextrose. During the insulin clamp, plasma glucose concentration was determined every 5 min. Plasma insulin con-

centration and 3-^{3}H-glucose specific activity were measured every 10–15 min. Continuous indirect calorimetry was done with a ventilated hood system (Deltatrac II, SensorMedics, Yorba Linda, Calif., USA) during the last 40 min of the basal and each insulin infusion step. Urine samples for determination of urea nitrogen excretion were obtained during the 3 h before and after the start of the insulin clamp.

NEFA turnover. [1-^{14}C] palmitate (New England Nuclear, Boston, Mass., USA) was utilized to measure plasma FFA turnover [23]. A primed (2.5 μCi)-continuous (0.1 μCi/min) infusion of 1-^{14}C-palmitate was started 180 min before the two-step insulin clamp and continued throughout the study. At –180 min, a 5 μCi bolus of [1-^{14}C] $NaHCO_3$ (New England Nuclear, Boston, Mass., USA) was given to prime the bicarbonate pool. ^{14}C-FFA plasma specific activity was determined at 5–10 min intervals during the last 30 min of basal period (–30 to 0 min) and during the last 30 min of each insulin clamp step.

NEFA oxidation. Plasma NEFA oxidation was calculated from the specific activity of expired CO_2 at 5–10 min intervals during the last 30 min of basal period (–30 to 0 min) and during the last 30 min of each insulin clamp step [23]. Expired air was bubbled through a CO_2 trapping solution (1 mol/l hyamine hydroxide/absolute ethanol: 0.1 % phenolphthalein, 3:5:1) that was titrated with 1 N HCl to trap 1 mmol of CO_2 per 3 ml of solution.

Assays. Plasma glucose concentration was measured using the glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, Calif., USA). Plasma insulin (Diagnostic Products Corporation, Los Angeles, Calif., USA) and C-peptide (Diagnostic Systems Laboratories, Webster, Tex., USA) concentrations were measured by radioimmunoassay. HbA_{1c} was measured by affinity chromatography (Biochemical Methodology, Drower 4350; Isolab, Akron, Oh., USA). Plasma NEFA concentrations were measured by an enzymatic calorimetric method (Wako Chemicals, Neuss, Germany). Plasma total cholesterol, HDL-cholesterol and triglyceride concentrations were measured enzymatically (Boehringer-Mannheim, Indianapolis, Ind., USA) on a Hitachi 704 autoanalyzer. LDL cholesterol was calculated from the Friedewald equation. Tritiated glucose specific activity was determined in deproteinized plasma samples. The 1-^{14}C-FFA specific activity was determined as previously described [23].

Calculations. Under steady state, postabsorptive conditions the rate of endogenous glucose appearance (Ra) equals the 3-^{3}H-glucose infusion rate (dpm/min) divided by the steady state plasma 3-^{3}H-glucose specific activity (dpm/mg). During the insulin clamp, non-steady conditions prevail and Ra was calculated from Steele's equation [24]. Endogenous glucose production (EGP) was calculated as the R_a minus the exogenous glucose infusion rate. Total glucose disposal (TGD) equals the sum of EGP plus the exogenous glucose infusion rate. The glucose metabolic clearance rate (MCR) equals the rate of TGD divided by the steady state plasma glucose concentration. Rates of glucose and lipid oxidation were calculated from oxygen consumption and carbon dioxide production data obtained from indirect calorimetry, using formulas described previously [25]. Non-oxidative glucose disposal, an index of glycogen formation, was calculated by subtracting the rate of glucose oxidation from the rate of TGD.

Plasma NEFA turnover was calculated as the rate of 1-^{14}C-palmitate infusion (dpm/min) divided by the steady-state plasma NEFA specific activity (dpm/μmol). Plasma NEFA concentration and specific activity were constant during the last 30 min of the basal period and during the last 30 min of each insulin clamp step. Therefore, all calculated rates of NEFA turnover pertain to steady-state conditions. Because fatty acids stored as triglycerides in adipose tissue represent the primary source of plasma NEFA [26], rates of NEFA turnover are expressed in terms of kilograms of fat mass. Because muscle and liver are the primary sites of NEFA oxidation [26], all NEFA and lipid oxidation rates are expressed as kilograms of fat free mass. The rate of oxidation of plasma NEFA in the basal state and during each insulin clamp step was calculated from ^{14}C radioactivity in expired CO_2 divided by the product of plasma NEFA specific activity and a factor k, which takes into account the incomplete recovery of labelled $^{14}CO_2$ from the bicarbonate pool [23]. The plasma NEFA oxidation rate (μmol/FFM kg · min) = ($^{14}CO_2$ specific activity X Vco_2)/(k X ^{14}C-FFA specific activity), where Vco_2 equals the total CO_2 production (in μmol/min) and k = 0.81.

Total body water was calculated from the mean plasma ^{3}H-water radioactivity at 90, 105, and 120 min after the intravenous bolus of ^3H_2O. Plasma tritiated water specific activity was calculated assuming that plasma volume represents 93 % of total volume. Fat free mass (FFM) was calculated by dividing total body water by 0.73 [27].

The area under the glucose, insulin, C-peptide, and NEFA curves during the OGTT were determined using the trapezoidal rule. The mean plasma glucose, insulin, C-peptide, and NEFA concentrations during the OGTT were calculated by dividing the area under the curve by 120 min. The insulinogenic index was calculated as the increment in plasma insulin divided by the increment in plasma glucose during the 0–30-min and 0–120-min time periods of the OGTT. From the plasma glucose and insulin concentrations during the OGTT, we also calculated a composite index of whole body insulin sensitivity [28].

Statistical analysis. Statistics were performed with StatView for Windows, v 5.0 (SAS Institute, Cary, N.C. USA). Values before and after treatment within each group (intragroup) were analysed using the paired Student's t test. Comparisons between groups (intergroup) were done using analysis of variance with Bonferroni/Dunn post hoc testing when appropriate. Pearson's correlations between continuous variables were used as a measure of association. A stepwise multiple linear regression analysis was done to examine the multiple correlations among variables. All data are presented as the means ± SEM. A p value of less than 0.05 was considered to be statistically significant.

Results

Patient characteristics. At baseline, the rosiglitazone and placebo-treated groups were similar in age (54 ± 2 vs 56 ± 2 y), gender (male/female: 7/8 vs 9/5), duration of diabetes (6 ± 3 vs 4 ± 1 y), body weight, BMI, fat mass, fat free mass, HbA_{1C} and fasting plasma glucose (FPG), insulin, C-peptide, and lipid concentrations (Table 1).

After 12 weeks of treatment, body weight, BMI, and fat mass increased in the rosiglitazone group ($p < 0.001$) and remained unchanged in the placebo group (Table 1). Fasting plasma glucose and HbA_{1c} decreased in the rosiglitazone group ($p < 0.01$) and the decrease was significantly different from the pla-

Table 1. Body weight and fat mass, parameters of glycaemic control, and plasma lipids before and after rosiglitazone or placebo treatment for 12 weeks

	Rosiglitazone ($n = 15$)		Placebo ($n = 14$)		p value for change (rosiglitazone vs placebo)
	before	after	before	after	
Body weight (kg)	86 ± 4	90 ± 4[b]	87 ± 5	87 ± 5	
change (%)	3.7 ± 0.8(4.4 %)		0 ± 0.4(0 %)		0.0003
BMI (kg/m^2)	30.0 ± 1.1	31.3 ± 1.1[b]	30.1 ± 1.0	30.1 ± 1.0	
change (%)	1.3 ± 0.3(4.4 %)		0 ± 0.1(0 %)		0.0004
Fat mass (kg)	33 ± 3	37 ± 3[b]	33 ± 3	33 ± 3	
change (%)	3.1 ± 0.6(11 %)		–0.1 ± 0.3(0 %)		0.0001
Fat free mass (kg)	53 ± 2	53 ± 2	54 ± 3	54 ± 3	
change (%)	0.6 ± 0.2(1 %)		0.1 ± 0.2(0 %)		0.07
FPG (mg/dl)	195 ± 11	150 ± 7[b]	188 ± 8	189 ± 11	
change (%)	–45 ± 8(–21 %)		0 ± 11(2 %)		0.003
HbA_{1c} (%)	8.7 ± 0.4	7.4 ± 0.3[b]	8.3 ± 0.4	8.7 ± 0.4	
change (%)	–1.3 ± 0.3(–14 %)		0.5 ± 0.3(7 %)		0.0001
FPI (μl/ml)	19 ± 3	15 ± 2	16 ± 3	15 ± 2	
change (%)	–4 ± 2(–15 %)		–1 ± 3(9 %)		
C-peptide (ng/ml)	3.0 ± 0.3	1.9 ± 0.2[a]	2.6 ± 0.4	2.1 ± 0.4	
change (%)	–1.1 ± 0.4(–25 %)		–0.5 ± 0.4(–9 %)		
Total cholesterol (mg/dl)	180 ± 9	195 ± 9	172 ± 5	169 ± 7	
change (%)	15 ± 8(10 %)		–3 ± 6(–2 %)		0.08
LDL (mg/dl)	108 ± 8	116 ± 7	108 ± 4	109 ± 7	
change (%)	8 ± 7(12 %)		1 ± 7(2 %)		
HDL (mg/dl)	34 ± 3	38 ± 3[a]	39 ± 3	37 ± 2	
change (%)	4 ± 2(16 %)		–3 ± 2(–5 %)		0.01
Triglycerides (mg/dl)	183 ± 15	181 ± 23	155 ± 33	203 ± 63	
change (%)	–2 ± 14(–2 %)		48 ± 32(13 %)		
NEFA (μEq/l)	735 ± 52	579 ± 49[b]	697 ± 41	778 ± 62	
change (%)	–156 ± 35(–20 %)		81 ± 62(15 %)		0.02

Data are means ± SEM
[a] $p < 0.05$ vs before rosiglitazone
[b] $p < 0.01$ vs before rosiglitazone

cebo group (Table 1). Fasting plasma insulin tended to decrease and fasting plasma C-peptide fell significantly from baseline in the rosiglitazone group, although these changes were not significantly different from placebo group. Fasting plasma HDL cholesterol increased significantly in the rosiglitazone group and remained unchanged in the placebo group (Table 1). Total cholesterol, LDL cholesterol, and triglyceride concentrations did not change significantly following rosiglitazone treatment. In the rosiglitazone group the fasting plasma NEFA concentration fell significantly from baseline ($p < 0.01$) and versus placebo ($p = 0.02$).

OGTT. Before treatment the plasma glucose, insulin, C-peptide, and NEFA concentrations during the OGTT were similar in rosiglitazone and placebo groups. After rosiglitazone treatment the mean plasma glucose and NEFA concentrations during the OGTT decreased significantly from baseline and the decrease was significantly different from placebo group (Table 2). There was no significant change in the mean plasma insulin and C-peptide concentrations or in the insulinogenic index (from 0 to 30 min or from 0–120 min) during the OGTT after rosiglitazone and placebo treatment. The insulin sensitivity index during the OGTT [28] increased from baseline in the rosiglitazone group ($p = 0.02$ vs placebo) (Table 2).

Euglycaemic insulin clamp: Plasma glucose and insulin. During the insulin clamp studies before treatment, the plasma glucose concentrations during basal period (175 ± 11 vs 162 ± 9 mg/dl) and during the first (110 ± 6 vs 97 ± 3 mg/dl) and second (91 ± 1 vs 89 ± 1 mg/dl) insulin clamp steps were similar in rosiglitazone and placebo groups. The steady state plasma insulin concentrations during the first (73 ± 6 and 65 ± 4 μU/ml) and second (350 ± 16 and 341 ± 23 μU/ml) steps of the insulin clamp also were similar in the rosiglitazone and placebo groups, respectively. The fasting plasma insulin concentration following treatment with rosiglitazone (14 ± 2 μU/ml) and placebo (14 ± 1 μU/ml) did not change from baseline. During the first and second insulin clamp steps done after treatment, the plasma glucose [rosig-

Table 2. Oral glucose tolerance test before and after rosiglitazone or placebo treatment for 12 weeks

	Rosiglitazone ($n = 15$)		Placebo ($n = 14$)		p value for change (rosi vs placebo)
	before	after	before	after	
Mean PG (mg/dl)	283 ± 12	236 ± 9[b]	293 ± 11	289 ± 13	
change (%)	–57 ± 9(–19 %)		–4 ± 13(–1 %)		0.002
Mean PI (μU/ml)	42 ± 8	41 ± 8	30 ± 4	28 ± 4	
change (%)	–1 ± 3(–5 %)		–1 ± 3(–3 %)		
Mean C-peptide (ng/ml)	5.2 ± 0.7	4.1 ± 0.6	4.3 ± 0.5	3.5 ± 0.6	
change (%)	–1.1 ± 0.7(–8 %)		–0.7 ± 0.6(–12 %)		
Mean NEFA (μEq/l)	561 ± 33	424 ± 35[b]	542 ± 43	631 ± 52	
change (%)	–137 ± 30(–24 %)		89 ± 47(23 %)		0.0003
Insulinogenic index (0–30 min)	0.26 ± 0.08	0.26 ± 0.08	0.12 ± 0.06	0.22 ± 0.07	
change (%)	–0.01 ± 0.06(–2 %)		0.10 ± 0.06(83 %)		
Insulinogenic index (0–120 min)	0.24 ± 0.06	0.34 ± 0.09	0.14 ± 0.04	0.14 ± 0. 03	0.09
change (%)	0.09 ± 0.05(38 %)		0.00 ± 0.03(0 %)		
Insulin sensitivity index	2.0 ± 0.2	2.9 ± 0.4[b]	2.6 ± 0.4	2.7 ± 0.4	
change (%)	0.9 ± 0.3(52 %)		0.1 ± 0.2(8 %)		0.02

Data are means ± SEM
Insulin sensitivity index = $10{,}000/\sqrt{(\text{FPG} \times \text{FPI}) \times (\text{mean PG} \times \text{mean PI})}$
[a] $p < 0.05$ vs before rosiglitazone
[b] $p < 0.01$ vs before rosiglitazone

litazone: 94 ± 1 (first step) and 89 ± 1 (second step) mg/dl; placebo: 106 ± 4 (first step) and 89 ± 1 (second step) mg/dl] and plasma insulin [rosiglitazone: 61 ± 3 (first step) and 323 ± 12 (second step) μU/ml; placebo: 69 ± 4 (first step) and 329 ± 18 (second step) μU/ml] concentrations were similar to those in the baseline insulin clamp study.

Basal endogenous glucose production (EGP). Basal EGP was similar in the rosiglitazone (3.26 ± 0.15 mg/kg FFM · min) and placebo (2.99 ± 0.09 mg/kg FFM · min) groups at the baseline (Table 3). After 12 weeks, basal EGP decreased in rosiglitazone group ($p < 0.05$) but did not change in the placebo group. The reduction in basal EGP after rosiglitazone was greater than placebo ($p = 0.01$) (Table 3).

Glucose metabolism during insulin clamp. During the baseline insulin clamp study EGP, total glucose disposal (TGD), and total body glucose metabolic clearance rate (MCR) in the first and second insulin clamp steps were similar in the rosiglitazone and placebo groups (Table 3). After 12 weeks of treatment, the reduction in EGP during the first ($p = 0.08$) and second ($p = 0.04$) insulin clamp steps was greater in the rosiglitazone than in the placebo group (Table 3). Total body glucose disposal during the first insulin clamp step increased slightly after rosiglitazone, and the glucose metabolic clearance rate rose ($p < 0.01$) (Table 3). During the second insulin clamp step total glucose disposal increased by 31 % ($p < 0.05$) after rosiglitazone treatment and this was paralleled by a similar rise in glucose metabolic clearance rate ($p < 0.05$ vs baseline and placebo) (Table 3). Both oxidative ($p < 0.05$) and non-oxidative glucose ($p = 0.05$) disposal during the second insulin clamp step increased after rosiglitazone and the increase in non-oxidative glucose disposal was greater than in the placebo group ($p < 0.05$) (Table 3).

Plasma NEFA concentration, turnover, oxidation. During the baseline study, plasma NEFA concentration, NEFA turnover, and NEFA oxidation under postabsorptive conditions and during the first and second insulin clamp steps were similar in rosiglitazone and placebo groups (Table 4). The fasting plasma NEFA concentration decreased significantly after rosiglitazone treatment ($p < 0.05$) (Table 4). There was a small, but statistically insignificant decline in the plasma NEFA concentration in the placebo group. After 12 weeks of rosiglitazone, the basal rate of NEFA turnover decreased compared to the pretreatment study ($p < 0.01$) and placebo group ($p < 0.05$) (Table 4). Similar results are obtained if the NEFA turnover data are expressed in terms of kilogram of FFM (rosiglitazone: 11.3 ± 1.1 to 10.0 ± 0.7, $p < 0.05$; placebo: 9.2 ± 0.9 to 8.9 ± 0.6, p = NS). During the first insulin clamp step, insulin-mediated suppression of plasma NEFA concentration was enhanced in the rosiglitazone group and did not change in the placebo group (Table 4). The NEFA turnover rate was also suppressed by 50–60 % in both insulin clamp steps in diabetic patients treated with rosiglitazone and placebo. Basal and insulin-mediated suppression of NEFA and lipid oxidation was similar in the rosiglitazone and placebo-treated groups before and after treatment (Table 4).

Table 3. Hepatic and whole body glucose metabolism during the two-step insulin clamp (insulin infusion rates = 40 and 160 mU/m^2 · min) studies done before and after 12 weeks of rosiglitazone or placebo treatment

	Rosiglitazone (n = 15)		Placebo (n = 14)		p value for change (rosiglitazone vs placebo)
	before	after	before	after	
Basal step (–30 to 0 min)					
Plasma glucose (mg/dl)	175 ± 11	132 ± 5[b]	162 ± 9	175 ± 11	
Plasma insulin (μU/min)	16 ± 2	14 ± 2	12 ± 2	14 ± 1	
EGP (mg/kg FFM · min)	3.26 ± 0.15	2.85 ± 0.11[a]	2.99 ± 0.09	3.14 ± 0.14	
change	–0.41 ± 0.16		0.15 ± 0.16		0.01
1st step (90–120 min)					
EGP (mg/kg FFM · min)	1.37 ± 0.15	1.03 ± 0.18	1.20 ± 0.13	1.33 ± 0.18	
change	–0.34 ± 0.20		0.13 ± 0.17		0.08
TDG (mg/kg FFM · min)	2.97 ± 0.14	3.26 ± 0.18	3.55 ± 0.38	3.64 ± 0.35	
change	0.29 ± 0.17		0.10 ± 0.12		
GOX (mg/kg FFM · min)	2.31 ± 0.16	2.75 ± 0.19	2.57 ± 0.19	2.64 ± 0.23	
change	0.44 ± 0.25		0.07 ± 0.22		
NOGD (mg/kg FFM · min)	0.69 ± 0.15	0.58 ± 0.13	1.02 ± 0.30	1.05 ± 0.28	
change	–0.12 ± 0.15		0.03 ± 0.19		
MCR (ml/kg FFM · min)	2.79 ± 0.19	3.54 ± 0.22 [b]	3.75 ± 0.46	3.50 ± 0.42	
change	0.75 ± 0.17		–0.25 ± 0.17		0.0003
2nd step (210–240 min)					
EGP (mg/kg FFM · min)	0.49 ± 0.14	0.29 ± 0.08	0.19 ± 0.09	0.39 ± 0.12	
change	–0.20 ± 0.13		0.20 ± 0.14		0.04
TDG (mg/kg FFM · min)	6.25 ± 0.56	8.21 ± 0.75[a]	7.54 ± 0.89	7.28 ± 0.88	
change	1.96 ± 0.78		–0.26 ± 0.29		0.01
GOX (mg/kg FFM · min)	3.64 ± 0.19	4.14 ± 0.25[a]	4.01 ± 0.25	4.04 ± 0.20	
change	0.50 ± 0.20		0.03 ± 0.21		
NOGD (mg/kg FFM · min)	2.61 ± 0.50	4.07 ± 0.56[c]	3.54 ± 0.76	3.32 ± 0.75	
change	1.46 ± 0.68		–0.21 ± 0.39		0.04
MCR (mg/kg FFM · min)	6.71 ± 0.59	9.24 ± 0.75[a]	8.53 ± 0.92	8.11 ± 1.04	
change	2.53 ± 0.86		–0.43 ± 0.33		0.004

Data are means ± SEM
[a] $p < 0.05$ vs before rosiglitazone
[b] $p < 0.01$ vs before rosiglitazone
[c] $p = 0.05$ vs before rosiglitazone

EGP, Endogenous glucose production rate; TGD, total body glucose disposal rate; GOX, glucose oxidation rate; NOGD, non-oxidative glucose disposal rate; MCR, total body metabolic clearance rate of glucose

Regression analysis. The change in FPG correlated positively with the changes in fasting plasma NEFA, mean plasma NEFA during OGTT, and basal EGP. The change in FPG correlated inversely with changes in glucose MCR during the first and second insulin clamp steps and with fat mass (Table 5). The change in mean plasma glucose during the OGTT correlated positively with changes in fasting plasma NEFA, mean plasma NEFA during the OGTT and EGP during basal period and the first step insulin clamp. The change in mean plasma glucose during the OGTT it correlated inversely with changes in glucose MCR during first and second insulin clamp steps and with fat mass (Table 5). Using backward stepwise multivariate analysis, the combined changes in fasting plasma NEFA, basal EGP, and glucose MCR during the first step insulin clamp step were the strongest predictors of the change in FPG concentration ($R = 0.79$, $p < 0.0001$). The combined changes in basal EGP and glucose MCR during the first step insulin clamp were the most important predictors of the change in mean plasma glucose during OGTT ($R = 0.77, p < 0.0001$).

A significant inverse relation was observed between the change in total body fat mass and the change in fasting plasma NEFA before and after treatment with rosiglitazone and placebo (Fig. 1). The fasting and first insulin clamp step plasma NEFA concentrations were positively related to basal EGP ($r = 0.30$, $p < 0.05$) and to EGP during the first insulin clamp step ($r = 0.26$, $p < 0.05$), respectively. The plasma NEFA concentration and glucose MCR during both the first ($r = –0.25$, $p = 0.08$) and second ($r = –0.34$, $p < 0.01$) insulin clamp steps were inversely related.

Discussion

These results indicate that 12 weeks of rosiglitazone (8 mg/day) treatment reduces the fasting plasma glucose concentration (by 21%), the postprandial plasma glucose excursion (by 19%) during OGTT, and HbA_{1c} (by 1.3%) in Type II diabetic patients. These improvements in glucose homeostasis could result from either increased insulin secretion or enhanced tissue sensitivity to insulin, and/or an improvement in the combined effects of hyperglycaemia plus hyperinsulinaemia to promote glucose metabolism.

Table 4. Non-esterified fatty acid metabolism during the two-step insulin clamp (insulin infusion rates = 40 and 160 mU/m^2 · min) before and after 12 weeks of rosiglitazone or placebo treatment

	Rosiglitazone (n = 15)		Placebo (n = 14)		p value for change (rosiglitazone vs placebo)
	before	after	before	after	
Basal step (–30 to 0 min)					
Plasma NEFA (μEq/l)	726 ± 44	611 ± 38[a]	704 ± 49	631 ± 35	
change	–115 ± 31		–72 ± 46		
NEFA TR (μEq/kg FM · min)	18.3 ± 1.5	15.5 ± 1.2[a]	15.7 ± 1.6	15.0 ± 0.9	
change	–0.28 ± 0.4		–0.8 ± 0.9		0.04
NEFA OX (μEq/kg FM · min)	2.6 ± 0.4	2.4 ± 0.3	2.1 ± 0.2	2.0 ± 0.2	
change	–0.2 ± 0.1		–0.1 ± 0.1		
Lipid OX (μEq/kg FM · min)	3.9 ± 0.4	4.2 ± 0.4	4.2 ± 0.3	4.0 ± 0.3	
change	0.3 ± 0.4		–0.2 ± 0.3		
1st step (90–120 min)					
Plasma NEFA (μEq/l)	241 ± 25	208 ± 23	227 ± 16	23 2 ± 23	
change	–32 ± 14		5 ± 15		0.08
NEFA TR (μEq/kg FM · min)	8.6 ± 1.3	8.1 ± 0.9	8.0 ± 0.6	7.5 ± 0.7	
change	–0.5 ± 0.9		–0.5 ± 0.8		
NEFA OX (μEq/kg FFM · min)	1.4 ± 0.3	1.3 ± 0.2	1.3 ± 0.1	1.1 ± 0.1	
change	–0.1 ± 0.2		–0.2 ± 0.1		
Lipid OX (μEq/kg FFM · min)	3.0 ± 0.3	2.4 ± 0.5	2.5 ± 0.3	2.5 ± 0.4	
change	–0.5 ± 0.5		0.1 ± 0.3		
2nd step (210–240 min)					
Plasma NEFA (μEq/l)	188 ± 20	164 ± 14	153 ± 7	176 ± 18	
change	–25 ± 15		24 ± 15		0.03
NEFA TR (μEq/kg FM · min)	8.1 ± 1.2	7.8 ± 1.0	6.7 ± 0.5	7.1 ± 0.7	
change	–0.3 ± 0.7		0.4 ± 0.7		
NEFA OX (μEq/kg FFM · min)	1.4 ± 0.2	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.5	
change	–0.1 ± 0.1		–0.1 ± 0.1		
Lipid OX (μEq/kg FFM · min)	1.6 ± 0.3	1.2 ± 0.4	1.2 ± 0.3	1.0 ± 0.4	
change	–0.5 ± 0.3		–0.2 ± 0.2		

Data are means ± SEM
[a] $p < 0.01$ vs before rosiglitazone
NEFA TR, non-esterified fatty acid turnover rate; NEFA OX, non-esterified fatty acid oxidation rate; Lipid OX, lipid oxidation rate; FM, fat mass (kg); FFM, fat free mass (kg)

Table 5. Summary of Pearson correlation coefficients for the change in fasting plasma glucose concentration and the change in mean plasma glucose concentration during the OGTT with the changes in selected variables

	ΔNEFA	ΔmNEFA	ΔbEGP	Δ1stEGP	Δ1stMCR	Δ2ndMCR	ΔFM
ΔFPG	0.534[b]	0.656[b]	0.542[b]	0.362	–0.662[b]	–0.489[b]	–0.419[a]
ΔmPG	0.490[b]	0.657[b]	0.579[b]	0.406[a]	–0.684[b]	–0.543[b]	–0.512[b]

[a] $p < 0.05$
[b] $p < 0.01$
Δ = Change between before and after rosiglitazone or placebo treatment. mPG, Mean plasma glucose concentration during the OGTT; mNEFA, mean plasma non-esterified fatty acid concentration during the OGTT; bEGP, basal endogenous glucose production; 1st EGP, endogenous glucose production during 1st insulin clamp step; 1st MCR, total body metabolic clearance rate of glucose during 1st insulin clamp step; 2nd MCR, total body metabolic clearance rate of glucose during 2nd insulin clamp step; FM, fat mass

After rosiglitazone, the fasting plasma insulin and C-peptide concentrations declined, while the mean plasma insulin and C-peptide concentrations and insulinogenic index (0–30 and 0–120 min) during the OGTT did not change. Conflicting results have appeared concerning the effect of thiazolidinediones on insulin secretion in Type II diabetics [1–3, 14–17]. Some studies have reported reduced plasma insulin and/or C-peptide concentrations [1, 2, 15, 16], while others found no change in plasma insulin/C-peptide concentrations [3, 14, 17]. We believe that the variable effect of thiazolidinediones on plasma insulin/C-peptide concentrations can be explained by two opposing effects: firstly, a decline in fasting and postprandial glucose concentrations, which reduce glucose toxicity and enhance beta-cell function [29], and, secondly, improved insulin sensitivity, which leads to a reduction in insulin secretion [29, 30]. Because no increase in plasma insulin and C-peptide concentrations or insulinogenic index following rosiglitazone therapy was observed, it is difficult to ascribe the improvement in glucose homeostasis to enhanced insulin secretion. However, rosiglitazone might still have an effect on the beta cells. Improved glucose tolerance without chan-

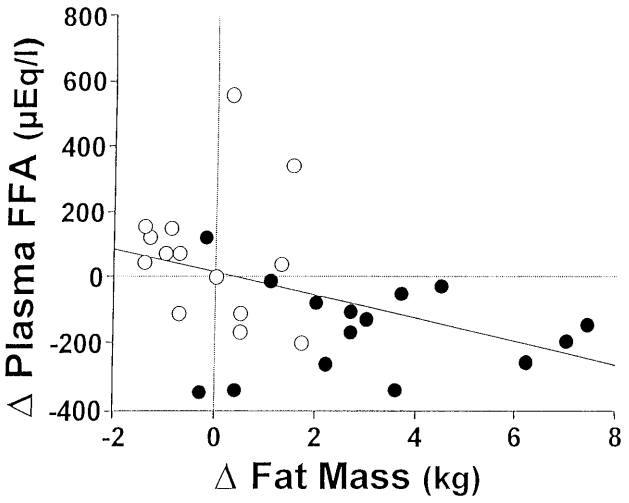

Fig. 1. Relation between the change in fasting plasma non-esterified fatty acid concentration (NEFA) and the change in fat mass (FM) before and after 12 weeks of rosiglitazone (●) and placebo (○) treatment; $r = -0.39$, $p < 0.05$

ges in the plasma insulin or C-peptide concentrations suggests an enhanced beta-cell function.

Following rosiglitazone therapy basal EGP decreased significantly (Table 3) and the reduction was strongly correlated with the reduction in fasting plasma glucose concentration ($r = 0.83$, $p < 0.0001$). These results are not surprising, because the basal rate of EGP is the primary determinant of the FPG (11, 29, 31). Stepwise regression analysis also demonstrated that the change in basal EGP is a strong independent predictor of the change in FPG concentration. It is noteworthy that in the initial clinical study [1] only those troglitazone-treated subjects who had reduced basal EGP experienced a decline in FPG and HbA_{1C} [1].

During the first and second insulin clamp steps, insulin-mediated suppression of EGP was significantly improved following rosiglitazone treatment (Table 3). The change in EGP during the first insulin clamp step was strongly correlated with the change in mean plasma glucose during the OGTT (Table 5). Because the steady-state plasma insulin concentrations during the first insulin clamp step were slightly lower after rosiglitazone treatment (see results section) and because the plasma insulin concentration is the major regulator of endogenous (hepatic) glucose production [11, 23, 29], we also measured the product of the EGP and SSPI concentration to provide a more precise measurement of hepatic insulin resistance. This index of hepatic insulin resistance declined in the rosiglitazone group (102 ± 13 to 64 ± 14 mg/kg FFM · min · μU/ml, $p < 0.05$) and rose slightly in the placebo group (80 ± 10 to 89 ± 13, p = NS), indicating enhanced hepatic sensitivity to insulin.

All previous studies that examined insulin sensitivity following thiazolidinedione (troglitazaone) treatment in Type II diabetic patients employed very high insulin infusion rates (80,120,300 mU/m^2 · min) that produced supraphysiologic plasma insulin concentrations [1–4]. In our study, the insulin infusion rate (40 mU/m^2 · min) during the first insulin clamp step was chosen specifically to cause a physiologic increase in the plasma insulin concentration (65–73 μU/ml). Under conditions of physiologic hyperinsulinaemia, rosiglitazone improved TGD by only 10%, and this did not reach statistical significance (Table 3). Because of the severity of insulin resistance, not all subjects achieved a plasma glucose concentration of 90–100 mg/dl during the first step insulin clamp. Therefore, we also calculated the MCR of glucose, which increased by 29% ($p < 0.01$) after rosiglitazone treatment. The improvement in glucose MCR was strongly correlated with both the decrease in FPG and the reduction in plasma glucose during the OGTT (Table 5). Stepwise regression analysis revealed that the change in MCR during the first insulin clamp step was an independent predictor of the decreases in FPG and mean plasma glucose during the OGTT. Our results show that thiazolidinediones enhance insulin sensitivity under physiologic conditions of hyperinsulinaemia. However, this insulin sensitizing effect is quite modest compared to the observed improvement in glucose homeostasis. Therefore, we also calculated the whole body insulin sensitivity index obtained from the plasma glucose and insulin concentrations during the OGTT [28]. This index, which takes into account the combined effects of both insulin (insulin sensitivity) and glucose (mass action effect of hyperglycaemia to improve glucose uptake) increased by 52% ($p < 0.01$) (Table 2), correlated with the change in glucose MCR during the first insulin clamp step ($r = 0.43$, $p < 0.05$), and rose much more than the increase in TGD (10%) or MCR of glucose (29%) during the first insulin clamp step. These results suggest that rosiglitazone has a stimulatory effect on glucose-mediated glucose uptake or on the combined effects of hyperglycaemia and hyperinsulinaemia to promote glucose disposal.

During the second insulin clamp step rosiglitazone improved TGD and glucose MCR by 32% and 38%, respectively, (Table 3) and this improvement in insulin sensitivity was correlated with the reductions in FPG and mean plasma glucose during OGTT (Table 5). The improvement in TGD during the second insulin clamp step was due to increased non-oxidative glucose disposal (Table 3), which primarily reflects glycogen synthesis. These results during the high dose euglycaemic insulin clamp are consistent with previous studies [1–4], which examined insulin sensitivity only in response to a pharmacologic increase in plasma insulin concentration in Type II diabetic subjects.

Rosiglitazone treatment resulted in a decrease in fasting plasma NEFA concentrations and a greater suppression of plasma NEFA during the OGTT (Table 1). The per cent decreases (~20%) in fasting and post-OGTT plasma NEFA concentrations were

similar to the per cent reduction in fasting and post-OGTT plasma glucose concentrations (Table 1) and a strong positive relation was observed between the change in fasting and post-OGTT plasma NEFA and glucose concentrations (Table 5). It is noteworthy that rosiglitazone treatment was associated with a weight gain of 3.7 kg, which was primarily accounted for by an increase in fat mass (3.1 kg). An increase in fat mass and a decrease in fasting plasma NEFA concentrations were observed in the majority of rosiglitazone-treated diabetic subjects (Fig. 1). These observations are consistent with previous results, which have consistently shown that thiazolidinediones reduce fasting plasma NEFA concentrations [1, 2, 5, 6, 15–19] and cause weight gain [6, 14–19]. The primary source of circulating plasma NEFA is from triglycerides stored in adipocytes [26] and fat cells in Type II diabetic patients are known to be resistant to insulin [23]. In rosiglitazone-treated diabetic patients, despite the consistent increase in fat mass (Fig. 1), fasting plasma NEFA (Fig. 1) and basal NEFA turnover decreased significantly despite a 15% reduction in the fasting plasma insulin concentration (Table 1). These results suggest that rosiglitazone improves adipose tissue sensitivity to the antilipolytic effect of insulin. Several mechanisms could explain the decrease in basal plasma NEFA concentrations and turnover rates: firstly, the direct insulin sensitizing effect of rosiglitazone on adipocytes [7, 32]; secondly, changes in fat topography, characterized by a decrease in visceral adipose mass and increases in subcutaneous fat mass [14, 33, 34]. It is known that visceral adipocytes are lipolytically more active than subcutaneous fat cells [35]; thirdly, remodelling of fat tissue characterized by apoptosis of differentiated, lipolytically more active large adipocytes in subcutaneous and/or visceral fat depots and differentiation of pre-adipocytes into small fat cells in subcutaneous fat depots [20, 32].

We also observed a significant, albeit modest, correlation between the fasting plasma NEFA concentration and basal EGP in all subjects before and after treatment ($r = 0.30, p < 0.05$). These results are consistent with previous publications [36] and suggest that the basal plasma NEFA concentration is an important determinant of the basal rate of EGP. They also suggest that the rosiglitazone-mediated decline in basal plasma NEFA concentration contributes to the decrease in basal EGP and improvement in glycaemic control. During both the first (physiologic plasma insulin = 60–70 μU/ml) and second (supraphysiologic plasma insulin = 320–350 μU/ml) insulin clamp steps, EGP was more effectively suppressed in the rosiglitazone than in the placebo group (Table 3). During the first insulin clamp step, plasma NEFA concentrations and EGP were correlated with each other ($r = 0.26$, $p < 0.05$). Inverse correlations were observed between the glucose MCR during the first ($r = –0.25, p = 0.05$) and second ($r = –0.34, p < 0.01$) insulin clamp steps and the plasma NEFA concentrations. The reduction in plasma glucose concentrations during the OGTT was strongly correlated with the reduction in post-OGTT plasma NEFA concentration. Taken collectively, these results suggest that the NEFA lowering effect of rosiglitazone contributes in part to the enhanced suppression of EGP by insulin and the increase in insulin-mediated glucose disposal by peripheral tissues. However, it does not seem likely that the reduction in plasma NEFA concentration can fully account for the improvement in hepatic/peripheral insulin sensitivity and glycaemic control following rosiglitazone treatment. Adipocytes can synthesize and secrete a number of proteins that exert local (paracrine) or distant (endocrine) effects on other tissues. Tumour necrosis factor α causes hepatic and peripheral tissue (muscle) resistance to insulin [37]. More recently, a new adipocyte-derived factor, resistin, that causes insulin resistance in vivo and in vitro in mice, has been reported [38]. Of note, rosiglitazone was shown to reduce resistin gene expression and circulating resistin concentrations. Rosiglitazone, by activation of the PPARγ receptor, could reduce the release from adipose tissue of NEFA, TNFα, resistin, and other, as of yet unidentified insulin antagonists, leading to improvements in hepatic/muscle insulin sensitivity and glycaemic control in Type II diabetic patients.

Consistent with previous clinical reports [15, 18, 19], plasma HDL cholesterol increased significantly during rosiglitazone treatment (Table 1). We did not observe any effect of rosiglitazone on plasma triglyceride or total/LDL cholesterol concentrations with rosiglitazone treatment.

In summary, this study examined the effect of any thiazolidinedione on NEFA metabolism and on hepatic/peripheral tissue insulin sensitivity under physiologic conditions of hyperinsulinaemia in Type II diabetic subjects. Our results indicate that rosiglitazone increases hepatic and peripheral (muscle) tissue sensitivity to insulin and decreases plasma NEFA concentrations and NEFA mobilization from adipose tissue, despite an increase in total body fat mass. The beneficial effects of rosiglitazone on NEFA metabolism are correlated with improved glucose metabolism and this observation partly explains how PPARγ activation in adipocytes improves insulin sensitivity in the liver and muscles. However, the correlations between the improvements in NEFA and glucose metabolism following rosiglitazone therapy are modest, suggesting that factors, in addition to NEFA metabolism, contribute to the drug's beneficial effects on glucose homeostasis.

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