

# Differential Effects of Rosiglitazone and Metformin on Adipose Tissue Distribution and Glucose Uptake in Type 2 Diabetic Subjects

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We evaluated the effects of rosiglitazone (4 mg b.i.d.) and metformin (1 g b.i.d.) monotherapy for 26 weeks on adipose tissue insulin-stimulated glucose uptake in patients ( $n = 41$ ) with type 2 diabetes. Before and after the treatment, glucose uptake was measured using 2-[<sup>18</sup>F]fluoro-2-deoxyglucose and positron emission tomography and adipose tissue masses were quantified using magnetic resonance imaging. Rosiglitazone improved insulin-stimulated whole-body glucose uptake by 44% ( $P < 0.01$  vs. placebo). Mean body weight was unchanged in the rosiglitazone group, while it decreased by 2.0 kg in the metformin group ( $P < 0.05$  vs. placebo). In visceral adipose tissue, glucose uptake increased by 29% (from  $17.8 \pm 2.0$  to  $23.0 \pm 2.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.05$  vs. placebo) in the rosiglitazone group but to a lesser extent (17%) in the metformin group (from  $16.2 \pm 1.5$  to  $18.9 \pm 1.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.05$  vs. baseline). Because the visceral adipose tissue mass simultaneously decreased with both treatments ( $P < 0.05$ ), no change was observed in total visceral glucose uptake per depot. Rosiglitazone significantly enhanced glucose uptake in the femoral subcutaneous area, either when expressed per tissue mass (from  $10.8 \pm 1.2$  to  $17.1 \pm 1.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.01$  vs. placebo) or per whole-fat depot ( $P < 0.05$  vs. placebo). In conclusion, metformin treatment resulted in improvement of glycemic control without enhancement of peripheral insulin sensitivity. The improved insulin sensitivity of the nonabdominal subcutaneous adipose tissue during treatment with rosiglitazone partly explains the enhanced whole-body insulin sensitivity and underlies the central role of adipose tissue for action of peroxisome proliferator-activated receptor  $\gamma$  agonist in vivo. *Diabetes* 52:283–290, 2003

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Received for publication 28 August 2002 and accepted in revised form 6 November 2002.

F.L. is a former employee of SmithKlineBeecham and GlaxoSmithKline (until 30 June 2001).

[<sup>18</sup>F]FDG, 2-[<sup>18</sup>F]fluoro-2-deoxyglucose; FFA, free fatty acid; MRI, magnetic resonance imaging; PET, positron emission tomography; PPAR, peroxisome proliferator-activated receptor; ROI, region of interest; TZD, thiazolidinedione.

**T**hiazolidinediones (TZDs) have been developed to alleviate peripheral insulin resistance. These agents are agonists of the peroxisome proliferator-activated receptor (PPAR)- $\gamma$ , a receptor subfamily regulating the genes controlling glucose homeostasis and lipid metabolism. As these receptors are predominantly expressed in adipose tissue (1), this has been suggested as their primary site of action (2,3). It has previously been shown that adipocyte glucose transport activity is improved after PPAR- $\gamma$  agonist treatment (2). Redistribution of fat stores has been suggested to explain the effects of TZDs. While the subcutaneous adipose tissue depots are expanding, the visceral fat mass decreases and adipocytes become smaller and more insulin sensitive (2,4). Furthermore, it has been shown that rosiglitazone reduces the serum free fatty acid (FFA) concentrations in patients with type 2 diabetes (5) and the lipolysis rate (as assessed by glycerol release) in human subcutaneous adipocytes exposed to chronic hyperinsulinemia (6). The effect on lipolysis has also been shown in vivo by microdialysis (5).

Along with skeletal muscle, adipose tissue is a site of peripheral insulin resistance in type 2 diabetes (7). The defective ability of insulin to inhibit lipolysis in adipose tissue leads to an increased FFA release. In addition, decreased glucose uptake in the adipose tissue may also contribute to elevated serum FFA levels. The latter is explained by a blunted conversion of glucose to triglycerides with a subsequent intracellular FFA accumulation. Compared with lean control subjects, the adipose tissue of obese patients has a reduced cellular content of GLUT4, which results in a reduced insulin-stimulated glucose transport capacity. We have recently shown that adipose tissue glucose uptake is impaired in obese subjects compared with lean subjects in vivo, using 2-[<sup>18</sup>F]fluoro-2-deoxyglucose ([<sup>18</sup>F]FDG) and positron emission tomography (PET) (8,9). Glucose transport capacity is further impaired in patients with type 2 diabetes (10).

It is unknown whether rosiglitazone treatment can improve adipose tissue glucose uptake in vivo in type 2 diabetic patients. The effects of TZDs on adipocyte glucose metabolism in humans have previously been studied in vitro using fat biopsies (2). As TZDs have also been shown to decrease the visceral fat depot mass (4) and to increase

TABLE 1  
Characteristics of the patients before and after treatment with rosiglitazone, metformin, or placebo

	Placebo		Metformin		Rosiglitazone	
	Baseline	26 weeks	Baseline	26 weeks	Baseline	26 weeks
Age (years)	58 ± 2		58 ± 2		58 ± 2	
Sex (M:F)	10:4		8:5		10:4	
Body weight	88.3 ± 2.6	88.4 ± 2.6	88.8 ± 3.1	86.8 ± 3.0*	83.7 ± 2.1	84.3 ± 2.4
BMI (kg/m <sup>2</sup> )	30.3 ± 1.2	30.3 ± 1.2	29.9 ± 1.1	29.2 ± 1.1*	29.1 ± 1.0	29.3 ± 1.1
Body fat (%)	30.7 ± 2.5	30.6 ± 2.4	31.8 ± 2.8	30.5 ± 2.5	30.4 ± 2.4	29.7 ± 2.4
Plasma glucose (mmol/l)	7.2 ± 0.3	7.2 ± 0.3	8.0 ± 0.5	6.8 ± 0.3†	7.2 ± 0.3	6.8 ± 0.3
HbA <sub>1c</sub> (%)	6.3 ± 0.1	6.1 ± 0.1	6.9 ± 0.2	6.2 ± 0.2‡	6.8 ± 0.2	6.5 ± 0.2§
Serum insulin (pmol/l)	60.4 ± 8.2	57.9 ± 5.7	70.2 ± 13.4	52.6 ± 7.2	51.4 ± 9.0	39.9 ± 2.5
Serum C-peptide (nmol/l)	0.86 ± 0.07	0.71 ± 0.04§	0.89 ± 0.10	0.65 ± 0.07§	0.78 ± 0.07	0.58 ± 0.04§
Serum total cholesterol (mmol/l)	4.6 ± 0.3	4.6 ± 0.2	4.5 ± 0.2	4.5 ± 0.2	4.8 ± 0.3	5.3 ± 0.3
Serum triglycerides (mmol/l)	1.9 ± 0.7	1.4 ± 0.3	1.2 ± 0.1	1.3 ± 0.2	1.7 ± 0.2	1.5 ± 0.2
Serum HDL cholesterol (mmol/l)	1.2 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Serum LDL cholesterol (mmol/l)	2.5 ± 0.2	2.7 ± 0.2	2.8 ± 0.2	2.6 ± 0.2	2.9 ± 0.2	3.5 ± 0.2
Serum FFAs (mmol/l)	0.61 ± 0.06	0.52 ± 0.05	0.51 ± 0.07	0.51 ± 0.05	0.59 ± 0.05	0.51 ± 0.06
Plasma lactate (mmol/l)	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.7 ± 0.1
M value (μmol · kg <sup>-1</sup> · min <sup>-1</sup> )	16.4 ± 2.9	17.5 ± 2.5	15.0 ± 2.0	15.3 ± 2.1	16.2 ± 2.3	23.3 ± 3.0¶

Data are means ± SE. Biochemical determinations were made from fasting samples. \**P* < 0.05, †*P* < 0.01, ‡*P* < 0.0001 vs. placebo; §*P* < 0.05 vs. baseline; ||*P* < 0.05, ¶*P* < 0.01 vs. placebo.

the size of subcutaneous adipose tissue mass (2) in parallel, it has been very difficult to estimate the effects of TZDs on regional adipose tissue glucose uptake based on the in vitro data.

Metformin is a widely used antihyperglycemic agent (11). It decreases insulin resistance and reduces hyperglycemia through a reduction of the hepatic glucose production in vivo in patients with type 2 diabetes (12,13). Metformin treatment results in a parallel weight reduction that is mainly due to a reduction in fat mass (12). Therefore, it is of interest whether metformin has an effect on adipose tissue glucose metabolism in vivo. Previous in vitro studies have shown that glucose uptake in adipose tissue is either increased (14) or unchanged (2) after treatment with metformin.

We have recently shown (15) that rosiglitazone improves skeletal muscle insulin-stimulated glucose uptake ~40% in the resting leg and ~100% during low-intensity exercise. During the same trial, we also measured the effects of rosiglitazone and metformin on abdominal and femoral subcutaneous and visceral adipose tissue insulin-stimulated glucose uptake using PET and [<sup>18</sup>F]FDG. This method was recently introduced and validated in our laboratory (8,9). We tested the hypothesis that rosiglitazone but not metformin enhances visceral and subcutaneous adipose tissue insulin-stimulated glucose uptake in vivo.

**RESEARCH DESIGN AND METHODS**

**Subjects.** Forty-four patients (aged 45–75 years, BMI 23–39 kg/m<sup>2</sup>) with type 2 diabetes, as defined by fasting plasma glucose (>7.0 mmol/l on at least two separate occasions) and presence of endogenous insulin production (fasting C-peptide >0.2 nmol/l), were included as described in detail earlier (15). Patients were excluded in case of fasting plasma glucose <6.1 or >10.0 mmol/l after the screening period, cardiac disease, blood pressure >160/100 mmHg, hepatic or renal diseases, symptoms of complications of diabetes, history of lactate acidosis, antidiabetic medication or oral corticosteroid treatment, and recent changes in antihypertensive medication or use of β-adrenergic blocking agents.

The nature, purpose, and potential risks of the study were explained to all subjects before they gave their written informed consent to participate. The ethics committee of the Hospital District of Varsinais-Suomi approved the

study. The study was conducted according to the principles of the Declaration of Helsinki.

Patients were randomized for sex and smoking. Three patients withdrew from the study; of these, one metformin patient was withdrawn due to symptoms of ischemic heart disease, and another metformin patient and one rosiglitazone patient were withdrawn because follow-up data were not received due to technical problems during the second PET study day. All analyses were based on the 41 patients who underwent PET scans both before and after the 26 weeks of treatment (Table 1).

**Study design.** All subjects first entered a 4-week run-in period during which they were advised to follow written instructions regarding a healthy low-fat diet. After the run-in period, the patients were randomly assigned to receive either 4 mg rosiglitazone b.i.d. (*n* = 14), 1 g metformin b.i.d. (*n* = 13), or placebo (*n* = 14) in a double-blind fashion. The subjects visited the study site at 2, 4, 8, 12, 18, and 26 weeks after the start of the treatment. The first [<sup>18</sup>F]FDG-PET study and magnetic resonance imaging (MRI) were performed after the 4-week run-in period before the double-blind period of the study, and the second PET study and MRI after the 26-week treatment period.

**Design for the PET study day.** The design of the [<sup>18</sup>F]FDG-PET study is shown in Fig. 1. Studies were performed after an overnight fast. Alcohol consumption and fatty meals were avoided for 3 days, and strenuous physical

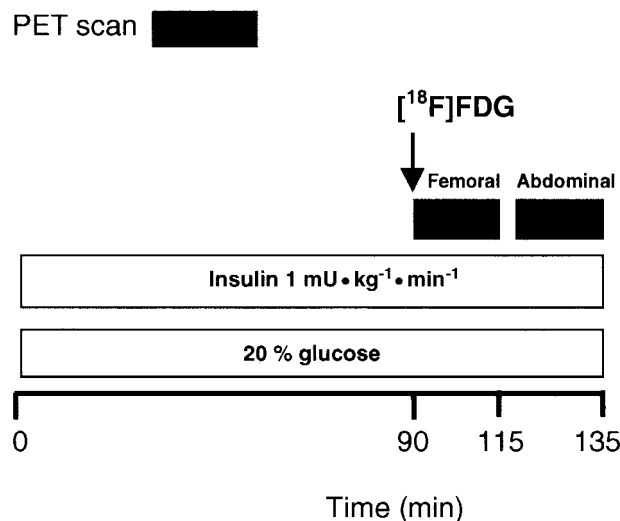


FIG. 1. Study design for [<sup>18</sup>F]FDG-PET studies. The arrow indicates the time point of positron emitting tracer ([<sup>18</sup>F]FDG) injection. Shaded rectangles denote the time period of dynamic scanning.

activity was not allowed for 48 h before the study. Two catheters were inserted, one to the antecubital vein of the left hand for infusion of glucose and insulin and injection of [ $^{18}\text{F}$ ]FDG and another to the radial artery in the right hand for blood sampling.

Each study consisted of a 140-min euglycemic-hyperinsulinemic ( $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) period. At 90 min, after steady-state glucose concentration had been reached, 0.18–0.19 GBq of [ $^{18}\text{F}$ ]FDG was injected intravenously and a 20-min dynamic scan of the femoral region was simultaneously started ( $2 \times 30 \text{ s}$ ,  $4 \times 60 \text{ s}$ , and  $3 \times 300 \text{ s}$  frames). Thereafter, an abdominal dynamic scan for 18 min ( $6 \times 180 \text{ s}$ ) was performed. Arterial blood samples for the measurement of plasma radioactivity were withdrawn once during each time frame and measured using an automatic gamma counter (Wizard 1480; Wallac, Turku, Finland).

Blood samples for the measurement of serum insulin and FFA and plasma lactate concentrations were taken during basal conditions and every 60 min. **Measurement of whole-body and adipose tissue glucose uptake.** Whole-body glucose uptake ( $M$  value;  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), as glucose disposal rate, was calculated using the euglycemic-hyperinsulinemic glucose infusion rates during 60–140 min (16).

[ $^{18}\text{F}$ ]FDG (half-life = 110 min) was synthesized as previously described (15). The specific radioactivity at the end of the synthesis was  $>75 \text{ GBq}/\mu\text{mol}$ , and the radiochemical purity exceeded 95%.

The subject was positioned supine in a 15-slice ECAT 931/08-tomograph (Siemens/CTI, Knoxville, TN) with the femoral or abdominal region within the gantry. Technical in-plane resolution was 6.5 mm and axial resolution 6.7 mm in the scanner.

Image acquisition and processing were performed as described earlier (15,17). Plasma and tissue time-activity curves for the adipose tissue were analyzed graphically to quantify the fractional rate of tracer uptake (8,18). Linear regression was used to determine the slope of the time-activity points between 2 and 18 min in the femoral area and between 27 and 41 min in the abdominal area after the [ $^{18}\text{F}$ ]FDG injection. The rate of regional glucose uptake was calculated by multiplying fractional [ $^{18}\text{F}$ ]FDG uptake by plasma glucose concentration divided by a lumped constant value of 1.14 in adipose tissue (8).

**MRI.** The abdominal region was imaged with a 0.23 T Outlook GP (Marconi Medical Systems, Vantaa, Finland) magnetic resonance imager using a body coil. Transverse T1-weighted field echo images with time repetition of 170 ms and time echo of 4 ms were obtained with the same pixel size ( $256 \times 256$ ) as the PET images. The level of the mid-slice and the upper and lower border of the area imaged were determined as previously described (9).

Adipose tissue masses in the abdominal region were measured at the level of intervertebral disc L2/L3, as earlier described by Abate et al. (19). In the femoral region, the adipose tissue area was measured in the middle of the thigh from an area 10 cm in length. The fat volume was converted to weight using an adipose tissue density of 0.9196 mg/ml.

The regions of interest (ROIs) were drawn on magnetic resonance images and located in subcutaneous (16 ROIs per analysis per patient) and visceral regions (12 ROIs per analysis per patient) in the abdominal area, as well as in subcutaneous adipose tissue of the femoral region (16 ROIs per analysis per patient). The ROIs were copied into the [ $^{18}\text{F}$ ]FDG images to cross-sectional slices from identical planes.

**Biochemical analyses.** Arterial plasma glucose was determined in duplicate by the glucose oxidase method (Analox GM9 Analyzer; Analox Instruments, London). HbA<sub>1c</sub> was measured by fast protein liquid chromatography (MonoS; Pharmacia, Uppsala, Sweden). Serum insulin and C-peptide were measured using a double-antibody fluoroimmunoassay (Autodelphia; Wallac). Plasma lactate and serum total cholesterol, triglycerides, and HDL cholesterol were measured using standard enzymatic methods (Boehringer Mannheim, Mannheim, Germany) with a fully automated analyzer (Hitachi 704; Hitachi, Tokyo). Serum LDL cholesterol was calculated according to the equation of Friedewald et al. (20). Serum FFAs were determined by an enzymatic method (ACS-ACOD Method; Wako Chemicals, Neuss, Germany).

**Anthropometric measurements.** Height and weight were measured by standard procedures. Body fat content was estimated with the bioelectric impedance method (Bioelectrical Impedance Analyzer; Akern, R.J.L. Systems, Florence, Italy). Whole-body fat mass was calculated using fat percentage and body weight.

**Statistical analyses.** Results are expressed as means  $\pm$  SE. Statistical calculations were performed using the SAS statistical program package (SAS Institute, Cary, NC). Differences between groups (drug effect) and within-group changes (time effect) and their interaction (drug  $\times$  time or drug  $\times$  visit [first clamp study versus second clamp study]) were compared using ANOVA for repeated measurements. Furthermore, if a significant interaction was found, one-way ANOVA and Tukey's honestly significant difference post hoc test were performed to test the changes between the groups. If a nonnormal

distribution of a variable was found, a nonparametric one-way analysis was concomitantly performed. Paired  $t$  test was used to test the changes within the groups or the differences between various fat depots. Spearman's correlation coefficients were calculated where appropriate.

## RESULTS

**Glycemic control.** At the time of randomization, the fasting glucose concentrations were similar among the three groups, although they tended to be higher in the metformin group. Metformin decreased fasting plasma glucose by 15% ( $P < 0.01$  vs. placebo) and HbA<sub>1c</sub> by 10% ( $P < 0.0001$  vs. placebo). In the rosiglitazone group, there was a trend toward improved glycemic control with decreased fasting plasma glucose ( $P = 0.10$ ) and HbA<sub>1c</sub> values ( $P < 0.05$  vs. baseline) (Table 1). Fasting serum insulin tended to decrease in the metformin ( $P = 0.07$ ) and rosiglitazone groups ( $P = 0.08$ ) (Table 1). C-peptide concentration decreased in all groups (Table 1). The effects of treatment on fasting serum lipid and FFA concentrations are shown in Table 1.

**Whole-body insulin sensitivity.** Rosiglitazone increased whole-body insulin sensitivity by 44%, the improvement of the glucose disposal rate being clearly superior to the effects of placebo ( $P < 0.01$ ) and metformin ( $P < 0.05$ ) (Table 1). Skeletal muscle insulin-stimulated glucose uptake was enhanced by rosiglitazone (from  $26.6 \pm 3.4$  to  $36.8 \pm 3.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.01$  vs. placebo) but was unchanged by metformin (from  $26.2 \pm 2.8$  to  $21.2 \pm 3.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and by placebo (from  $24.2 \pm 2.8$  to  $24.1 \pm 3.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) (15).

**Metabolic characteristics during the clamp studies.** During the first (before treatment) euglycemic-hyperinsulinemic clamp, steady-state plasma glucose averaged  $5.2 \pm 0.1 \text{ mmol/l}$  in the placebo,  $5.4 \pm 0.1 \text{ mmol/l}$  in the metformin, and  $5.2 \pm 0.1 \text{ mmol/l}$  in the rosiglitazone group (NS between the groups). After treatment, similar steady-state glucose concentrations were recorded in all groups. Serum insulin concentrations were 20% lower during the clamp study after treatment than before in both the metformin and the rosiglitazone groups (before and after treatment:  $529 \pm 24$  and  $426 \pm 15 \text{ pmol/l}$ , respectively, in the metformin group and  $518 \pm 31$  and  $425 \pm 21 \text{ pmol/l}$ , respectively, in the rosiglitazone group,  $P < 0.01$  within the groups). Moreover, serum FFAs during hyperinsulinemia were suppressed by 50% in the rosiglitazone group ( $P < 0.01$  vs. baseline).

**Body weight and adipose tissue mass.** Metformin decreased the mean body weight by 2.0 kg ( $P < 0.05$  vs. placebo), while the body weight remained unchanged by placebo and rosiglitazone. Similarly, BMI decreased significantly in the metformin group ( $P < 0.05$  vs. placebo) (Table 1 and Fig. 2). Within the metformin group, both abdominal subcutaneous (from  $5.3 \pm 0.6$  to  $4.9 \pm 0.5 \text{ kg}$ ) and intra-abdominal fat masses (from  $2.5 \pm 0.3$  to  $2.2 \pm 0.2 \text{ kg}$ ) decreased significantly ( $P < 0.05$ ) (Fig. 3). In the rosiglitazone group, the visceral fat mass also decreased significantly (from  $2.3 \pm 0.3$  to  $2.0 \pm 0.2 \text{ kg}$ ,  $P < 0.05$  vs. placebo) (Fig. 3), whereas the abdominal subcutaneous fat depot remained essentially unchanged (Fig. 3).

**Adipose tissue glucose uptake (Fig. 3).** In all subjects, the rate of insulin-stimulated glucose uptake ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was approximately twice as high in visceral than abdominal subcutaneous adipose tissue ( $P < 0.0001$ ,

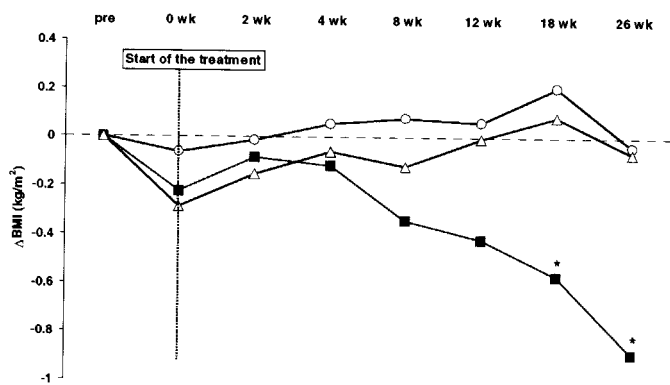


FIG. 2. The change in the BMI during the 26 weeks of treatment. The basal (pre) BMI was similar among the groups, and the dashed line indicates the start of the treatment. ○, placebo; ■, metformin; △, rosiglitazone. \**P* < 0.05 for the change vs. placebo.

paired *t* test) (Fig. 3). After treatment, there was a trend for abdominal subcutaneous adipose tissue glucose uptake to increase in both the metformin (from  $6.3 \pm 0.9$  to  $8.0 \pm 0.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , *P* = 0.12) and rosiglitazone groups (from  $8.8 \pm 1.0$  to  $11.9 \pm 2.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , *P* = 0.15). In the rosiglitazone group, the regional insulin-stimulated glucose uptake was increased by 29% in visceral adipose tissue (from  $17.8 \pm 2.0$  to  $23.0 \pm 2.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , *P* < 0.05 vs. placebo and *P* < 0.01 vs. baseline) and by 17% in the metformin group (from  $16.2 \pm 1.5$  to  $18.9 \pm 1.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ; *P* < 0.05 vs. baseline and NS vs. placebo). Furthermore, in femoral subcutaneous adipose tissue, rosiglitazone increased the glucose uptake rate by 58% (from  $10.8 \pm 1.2$  to  $17.1 \pm 1.7 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , *P* < 0.01 vs. placebo), whereas no change was observed in the metformin group (from  $10.9 \pm 1.1$  to  $12.0 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ).

**Glucose uptake in adipose depots.** By multiplying the regional adipose tissue glucose uptake by the regional adipose tissue mass, the glucose uptake could also be expressed per different adipose depots. In the abdominal area, no statistically significant treatment-induced changes could be observed by either metformin or rosiglitazone compared with placebo or baseline values (Fig. 3). However, in the femoral area, rosiglitazone significantly enhanced the subcutaneous adipose tissue glucose uptake (by 45%, *P* < 0.0001 vs. baseline and *P* < 0.05 vs. placebo) (Fig. 3).

**Relationship between adipose tissue, skeletal muscle, and whole-body glucose uptake.** Both at baseline and after treatment, visceral adipose tissue glucose uptake rate per kilogram correlated inversely with visceral fat mass in the pooled patient population (*r* = -0.36, *P* < 0.05 before and *r* = -0.34, *P* < 0.05 after the treatment). Furthermore, after treatment with rosiglitazone, the femoral subcutaneous adipose tissue glucose uptake rate per kilogram correlated inversely with the femoral fat mass (*r* = -0.53, *P* = 0.05).

Before the treatment was initiated, whole-body glucose uptake correlated with both visceral (*r* = 0.40, *P* = 0.01) and femoral subcutaneous fat glucose uptake (*r* = 0.37, *P* = 0.02), but not with abdominal subcutaneous uptake rate (Fig. 4). Similarly, visceral (*r* = 0.47, *P* = 0.002) and femoral subcutaneous adipose tissue glucose uptake (*r* = 0.49, *P* = 0.001) correlated with skeletal muscle glucose uptake. After treatment with rosiglitazone, whole-body glucose uptake correlated with glucose uptake rates in visceral (*r* = 0.80, *P* = 0.0005), abdominal (*r* = 0.54, *P* = 0.05), and femoral subcutaneous adipose tissue glucose uptake rates (*r* = 0.85, *P* = 0.0001) (Fig. 4). In concert with this, skeletal muscle glucose uptake correlated with visceral (*r* = 0.77, *P* = 0.001) and femoral subcutaneous adipose tissue glucose uptake (*r* = 0.80, *P* = 0.0006) after rosiglitazone treatment. No such correlations were found in the metformin group.

The relative contribution of the abdominal fat compartments to the total glucose uptake in the whole body was  $8 \pm 1\%$  before and remained essentially the same after the treatment, although a slight decrease was observed in the rosiglitazone group ( $6 \pm 1\%$ , NS).

**DISCUSSION**  
This study shows that simultaneously with the improvement of the whole-body insulin sensitivity, rosiglitazone enhances insulin-stimulated glucose uptake rate ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in intra-abdominal and femoral subcutaneous adipose tissue in patients with type 2 diabetes. Because visceral fat mass decreased during treatment with rosiglitazone, the net effect on glucose uptake is neutral. However, a significant increment of the net adipose tissue

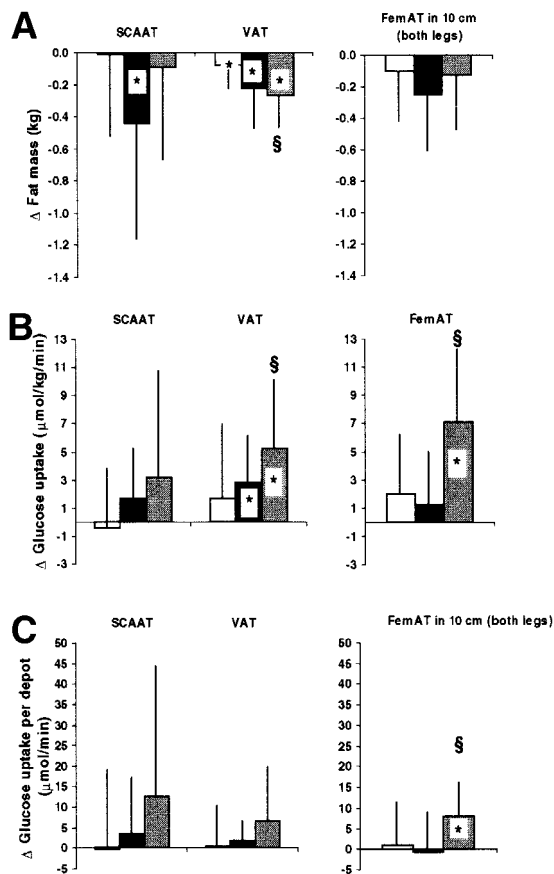


FIG. 3. The change in fat mass (A), the change in glucose uptake in adipose tissue per tissue weight (B), and the change in glucose uptake in whole-fat depot (C). Vertical bars denote SD. □, placebo; ■, metformin; ▨, rosiglitazone. FemAT, femoral subcutaneous adipose tissue; SCAAT, subcutaneous abdominal adipose tissue; VAT, visceral adipose tissue. §*P* < 0.05 for the change vs. placebo. Asterisks (\*) shown within the columns are for the changes (*P* < 0.05) within the group.

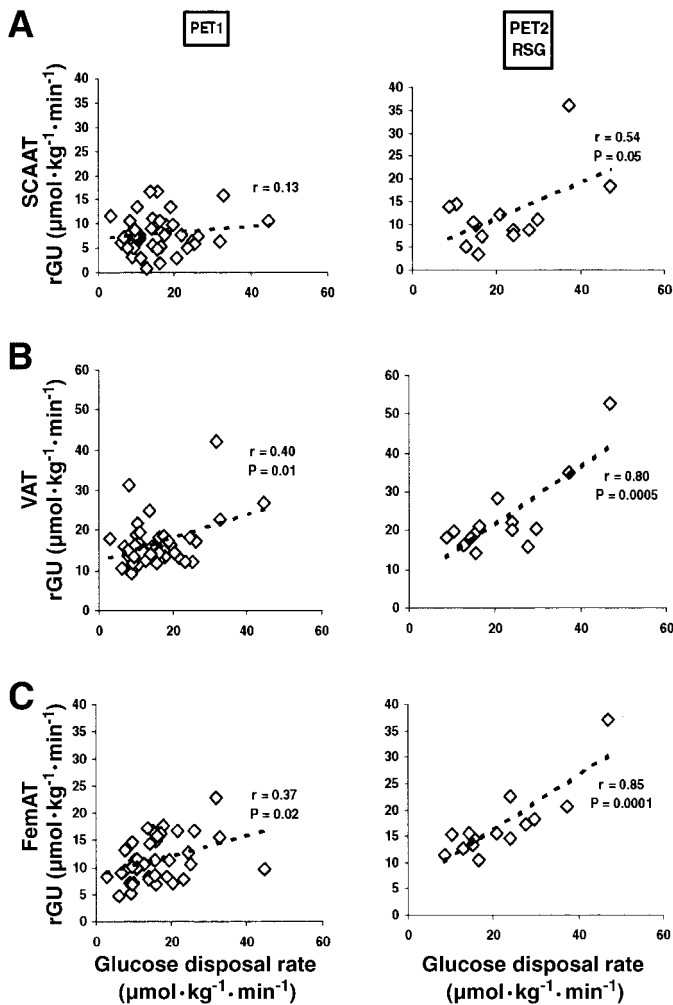


FIG. 4. Correlations between regional adipose tissue glucose uptake rates (rGU) per tissue weight and whole-body glucose disposal rates. Scatter plots for subcutaneous abdominal adipose tissue (SCAAT) (A), visceral adipose tissue (VAT) (B), and femoral subcutaneous adipose tissue (FemAT) (C). PET1, first PET study at baseline; PET2, second PET study after 26 weeks of treatment; RSG, rosiglitazone group.

glucose uptake was observed in femoral subcutaneous fat. Rosiglitazone improved glycemic control as assessed by HbA<sub>1c</sub> level. Metformin had no effect on either whole-body insulin sensitivity or subcutaneous adipose tissue insulin sensitivity in patients with mild type 2 diabetes, while its effect on the visceral adipose tissue glucose uptake may be due to a reduction of the visceral fat mass. Nevertheless, even if metformin has no effect on insulin sensitivity, it improves glycemic control due to its well-known inhibitory effect on hepatic glucose production (12).

We have recently applied the [<sup>18</sup>F]FDG-PET method for measurement of adipose tissue glucose uptake rate in obese and nonobese subjects in vivo (8,9). When PET is combined with [<sup>18</sup>F]FDG, quantification of glucose metabolism is performed directly in adipose tissue, thus avoiding any confounding effects caused by local catheters. Moreover, this method currently seems to be the only alternative for noninvasive in vivo quantification of metabolism in visceral adipose tissue. In accordance with our earlier study (9), visceral adipose tissue insulin-stimulated glucose uptake rates ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) were twice as high as in abdominal subcutaneous fat in all patients with type

2 diabetes. This is consistent with earlier in vitro studies in which visceral adipose tissue was shown to contain a larger number of adipocytes per gram of tissue than subcutaneous fat. The visceral adipocytes are also smaller than the subcutaneous ones (21), and insulin receptor expression is higher in the former depot (22), suggesting facilitated glucose uptake in intra-abdominal adipose tissue.

Body weight decreased by metformin as previously demonstrated (12,23). The most prominent mean decrease in fat mass was found in the abdominal subcutaneous fat depot, which is consistent with earlier data (23). Metformin increased the insulin-stimulated glucose uptake rate per kilogram within the group in visceral adipose tissue ( $P < 0.05$ ) (Fig. 3) but not in subcutaneous fat. In earlier in vitro studies, only subcutaneous adipocyte glucose uptake was investigated and shown to be either increased (14) or unchanged (2) after treatment with metformin. The results of the current study suggest that the contribution of adipose tissue insulin-stimulated glucose uptake on the antihyperglycemic effect of metformin is not important.

Weight increase has repeatedly been reported after treatment with rosiglitazone (24–26). However, one recent rosiglitazone trial reported no change in subject body weight after 3 months of rosiglitazone treatment (5), in line with the present study. The reasons for this lack of weight gain may be related to the patient population selected, which had mild or newly diagnosed disease. Furthermore, the written diet instructions given in the run-in phase of the trial may have contributed to this finding. Regarding fat distribution, rosiglitazone has been reported to result in a redistribution of visceral fat depot toward subcutaneous depots (4,27–29). Accordingly, the intra-abdominal fat mass decreased after treatment with rosiglitazone in the current study (Fig. 3). Body fat percentage was decreased in 10 of 14 rosiglitazone-treated patients, but only half of them lost their whole-body weight. When we estimated lean body mass using fat percentage and body weight, it tended to increase ( $P = 0.08$ ) in the rosiglitazone group. This might suggest an increment of muscle tissue mass or fluid retention. None of these patients had clinical symptoms or signs of fluid retention, although it cannot be totally excluded.

Treatment with rosiglitazone increased insulin-stimulated adipose tissue glucose uptake per kilogram in the visceral and the femoral subcutaneous regions. This finding corroborates earlier in vitro results, where rosiglitazone was shown to increase glucose uptake in 3T3-L1 adipocytes (30,31). Accordingly, glucose transport activity has been shown to improve after TZD treatment (2). The cellular basis for these effects may at least partly be due to changes in glucose transport proteins. Rosiglitazone has been shown to enhance adipose tissue glucose uptake rates by normalizing the GLUT4 protein content in adipose tissue and to increase the GLUT1 protein content in skeletal muscle and fat (32).

An inverse correlation was observed between the circulating FFAs during the hyperinsulinemic clamps and the glucose uptake rates ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in both visceral and femoral subcutaneous fat in the pooled data ( $r = -0.53$ ,  $P < 0.001$  and  $r = -0.58$ ,  $P < 0.0001$ , respectively).

This is in accordance with our finding that glucose uptake rate increased in the visceral and femoral adipose tissue by rosiglitazone because the steady-state FFA concentrations were concomitantly suppressed (by 50%) when compared with the baseline clamp examination. Suppression of the circulating FFAs by rosiglitazone during hyperinsulinemia has been shown to be due to both a more pronounced suppression of FFA release from adipose tissue into the bloodstream and an increased FFA clearance (33). We did not observe a significant reduction in fasting plasma FFAs, as shown in other studies (24–26). This discrepancy may be due to the better glycemic control of the patients at baseline in the current study. The average fasting plasma glucose tended to be decreased ( $P = 0.10$ ) by rosiglitazone, in concert with the trend of decreased fasting FFAs ( $P = 0.14$ ).

To evaluate the influence of treatment in the three groups on whole-fat depots, data derived from [ $^{18}\text{F}$ ]FDG-PET and MRI were combined. Since the glucose uptake rate ( $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was increased simultaneously with the decreased adipose tissue weight, the net uptake ( $\mu\text{mol}/\text{min}$ ) in visceral fat depot was similar between the groups. Inverse associations were observed between visceral fat mass and regional glucose uptake in the pooled data after the treatment ( $r = -0.50$ ,  $P < 0.05$ ), as well as between the changes in visceral fat mass and the increment in glucose uptake rate by metformin ( $r = -0.66$ ,  $P < 0.05$ ). The average net glucose uptake in abdominal subcutaneous fat depot ( $\mu\text{mol}/\text{min}$ ) was slightly but not significantly enhanced by rosiglitazone. In contrast to abdominal fat depots, insulin-stimulated glucose uptake was clearly enhanced by rosiglitazone in the femoral subcutaneous depot. In addition to the enhancement of glucose transport mechanisms (32), the activated adipogenesis in the femoral subcutaneous fat might be partly responsible for the increased glucose uptake in this region. Subcutaneous preadipocytes have been shown to be prone to the adipogenesis-promoting effect of rosiglitazone when compared with omental preadipocytes in vitro (33a). The differences between the effect of rosiglitazone on abdominal and femoral subcutaneous adipose tissue glucose uptake may be due to physiological site differences in these depots. In vitro and ex vivo studies (34) show that in obese women, basal and insulin-stimulated rates of glucose oxidation are twice as great in femoral as in abdominal fat. Further, insulin binding during insulin stimulation ( $\geq 15$  pmol/ml) is higher in femoral than abdominal subcutaneous adipocytes (34).

Rosiglitazone treatment resulted in a 44% improved whole-body insulin sensitivity and a 38% improved insulin sensitivity in resting skeletal muscle tissue (15). These findings are in concordance with recent publications (35,36) in which a 12–77% improvement of the glucose infusion rate and an ~30% improvement of the insulin-stimulated diaphragm or splanchnic glucose uptake rate have been reported. Since the steady-state insulin concentrations were lower during the second than first clamp examinations in both the metformin and the rosiglitazone groups, we may have actually underestimated the effect of treatment on whole-body insulin sensitivity. Surprisingly, we found that metformin had no effect on whole-body insulin sensitivity despite significantly improved glycemic

control and reduced body weight. Metformin has been shown to decrease insulin resistance and reduce hyperglycemia through effects on hepatic glucose production in vivo in hyperglycemic patients with type 2 diabetes (12,13). In the present study, the insulin concentrations achieved during clamping condition presumably suppressed hepatic glucose production (37,38). In accordance with the current results, metformin has also been reported not to change whole-body insulin sensitivity (39,40). The metformin effect on glycemic control may, thus, be due to a decrease in hepatic glucose production that was not assessed during the prevailing clamping conditions. There are also numerous studies reporting improved peripheral sensitivity by metformin treatment (41–44). The discrepancy in results may, at least in part, be due to differences in glycemic control among the investigated patient groups. It should be noted that peripheral sensitivity may secondarily be improved in patients with poor metabolic control when treated with metformin. In the present study, the prevailing HbA<sub>1c</sub> levels indicated that glycemic control at baseline was good enough to prevent glucose toxicity (45).

Although the major proportion of the insulin-mediated whole-body glucose uptake occurs in skeletal muscle, a significant relationship was found between whole-body glucose disposal and adipose tissue glucose uptake rates in vivo. This association was especially strong in the visceral adipose tissue ( $r = 0.80$ ,  $P < 0.001$ ) and the femoral subcutaneous adipose tissue ( $r = 0.85$ ,  $P < 0.001$ ) (Fig. 4) in patients treated with rosiglitazone. Earlier, a strong correlation between whole-body glucose disposal and adipocyte glucose transport was demonstrated in animals (7), and a similar tendency to an improvement of fat cell glucose transport was reported in humans after treatment with troglitazone (2).

The total amount of glucose taken up by the abdominal adipose depots was 4% in subcutaneous and 4% in visceral region of the whole-body glucose disposal rate. In the femoral region, the glucose uptake was assessed for the restricted area only (over a length of 10 cm in both legs) and the relative proportion of whole-body insulin-stimulated glucose disposal was 2%. Assuming the rest of the nonabdominal adipose tissue consists mainly of subcutaneous adipose tissue, with glucose uptake rates per kilogram tissue similar to abdominal subcutaneous adipose tissue, the average glucose uptake in the total body adipose tissue would be ~22–26% during the clamp. This finding is in accordance with earlier data (up to 20% in morbidly obese subjects) (46,47). In healthy nondiabetic young obese men, the adipose tissue proportion of the whole-body glucose uptake was 13% (9). Therefore, although we may have slightly underestimated the whole-body glucose disposal rate, the proportion of glucose that was taken up by the adipose tissue in the whole body in the type 2 diabetic patients in the present study was notable.

In summary, rosiglitazone and metformin treatment have different effects on adipose tissue insulin-stimulated glucose metabolism in patients with drug-naïve, newly diagnosed, and/or diet-treated type 2 diabetes. Metformin treatment decreases adipose tissue mass but has no net effect on adipose tissue insulin-stimulated glucose uptake. Rosiglitazone also decreases visceral fat mass, while the

net uptake rate remains unchanged. These data also clearly show that subcutaneous adipose tissue insulin-stimulated glucose metabolism is differently affected by rosiglitazone, depending on the site. Subcutaneous adipose tissue glucose uptake is particularly enhanced in nonabdominal areas by rosiglitazone. This positive effect on adipose tissue insulin sensitivity indirectly, i.e., via suppression of FFAs by insulin, contributes to the parallel improvement in skeletal muscle and whole-body insulin sensitivity.

#### ACKNOWLEDGMENTS

This study was financially supported by grants from the Academy of Finland (P.N.), the Novo Nordisk Foundation (P.N. and P.L.), the Finnish Diabetes Research Society (K.A.V., K.H., and P.N.), the Finnish Cultural Foundation (K.A.V.), the Research and Science Foundation of Farnos (K.A.V.), the Swedish Research Council (P.L.), the Swedish Diabetes Association (P.L.), and GlaxoSmithKline.

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