Effects of Rosiglitazone and Metformin on Liver Fat Content, Hepatic Insulin Resistance, Insulin Clearance, and Gene Expression in Adipose Tissue in Patients With Type 2 Diabetes

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Both rosiglitazone and metformin increase hepatic insulin sensitivity, but their mechanism of action has not been compared in humans. The objective of this study was to compare the effects of rosiglitazone and metformin treatment on liver fat content, hepatic insulin sensitivity, insulin clearance, and gene expression in adipose tissue and serum adiponectin concentrations in type 2 diabetes.

A total of 20 drug-naïve patients with type 2 diabetes (age 48 ± 3 years, fasting plasma glucose 152 ± 9 mg/dl, BMI 30.6 ± 0.8 kg/m²) were treated in a double-blind randomized fashion with either 8 mg rosiglitazone or 2 g metformin for 16 weeks. Both drugs similarly decreased HbA1c, insulin, and free fatty acid concentrations. Body weight decreased in the metformin (84 ± 4 vs. 82 ± 4 kg, P < 0.05) but not the rosiglitazone group. Liver fat (proton spectroscopy) was decreased with rosiglitazone by 51% (15 ± 3 vs. 7 ± 1%, 0 vs. 16 weeks, P = 0.003) but not by metformin (13 ± 3 to 14 ± 3%, NS). Rosiglitazone (16 ± 2 vs. 20 ± 1 ml · kg⁻¹ · min⁻¹, P = 0.02) but not metformin increased insulin clearance by 20%. Hepatic insulin sensitivity in the basal state increased similarly in both groups. Insulin-stimulated glucose uptake increased significantly with rosiglitazone but not with metformin. Serum adiponectin concentrations increased by 123% with rosiglitazone but remained unchanged during metformin treatment. The decrease of serum adiponectin concentrations correlated with the decrease in liver fat (r = −0.74, P < 0.001). Rosiglitazone but not metformin significantly increased expression of peroxisome proliferator–activated receptor-γ, adiponectin, and lipoprotein lipase in adipose tissue. In conclusion, rosiglitazone but not metformin decreases liver fat and increases insulin clearance. The decrease in liver fat by rosiglitazone is associated with an increase in serum adiponectin concentrations. Both agents increase hepatic insulin sensitivity, but only rosiglitazone increases peripheral glucose uptake. Diabetes 53:2169–2176, 2004

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ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; FFA, free fatty acid; LPL, lipoprotein lipase; PPAR, peroxisome proliferator–activated receptor.

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Both rosiglitazone and metformin are widely used antidiabetic agents for which mechanisms of action are still incompletely understood. Zhou et al. (1) recently showed that, in rats, the elusive target for metformin is stimulation of AMP-activated protein kinase (AMPK), which is accompanied by inhibition of lipogenesis and a decrease in hepatic steatosis. Consistent with this proposal, metformin was, in an uncontrolled study, suggested to decrease transaminases in patients with nonalcoholic steatohepatitis (2). Several other studies in humans have also identified the liver as the primary target of metformin action (3–5), but there are no data examining whether hepatic insulin sensitization by metformin is associated with a change in liver fat content.

PPAR-γ agonists such as pioglitazone and rosiglitazone improve glycemic control, decrease serum insulin concentrations, and appear to enhance whole-body insulin sensitivity (6–9). The latter is due to enhanced insulin action in both peripheral tissues and the liver (6,8,9). Direct comparison of troglitazone and metformin suggested that stimulation of peripheral glucose disposal is the primary mechanism of troglitazone action (3). In uncontrolled studies, rosiglitazone (7,10) and pioglitazone (8) have been shown to decrease liver fat but not intramyocellular lipid (10) content in humans. This contrasts data in a mouse model, in which rosiglitazone treatment doubled the size of the liver in animals with a fatty liver at baseline (11). A decrease in liver fat content might be expected to increase hepatic sensitivity (12,13). Possibly, a decrease in hepatic fat content might also improve insulin clearance (14,15). There are no data comparing effects of rosiglitazone and metformin on liver fat content and insulin action on glucose production and utilization in humans. It is also unknown whether changes in insulin clearance contribute to the decrease in fasting serum insulin concentrations during PPAR-γ agonist therapy.

The nuclear receptor PPAR-γ is predominantly expressed in adipose tissue and to a lesser extent in liver and muscle (16). The mechanism by which PPAR-γ agonists enhance insulin sensitivity of glucose production and utilization is unclear. One possibility is that PPAR-γ effects in muscle and the liver are indirect and result from...
modification of gene expression in adipose tissue. In vitro, in adipocytes (17,18), C/EBP-α’ fibroblasts (19), and mouse models (20), PPAR-γ agonists increase adiponectin expression (18), which in mice has insulin-sensitizing effects, especially in the liver (21,22). The relevance of these observations to human physiology is unclear because there are no data on effects of PPAR-γ agonists on gene expression in adipose tissue.

In the present study, we wished to directly compare, in a randomized double-blind fashion, effects of rosiglitazone and metformin treatment of drug-naïve patients with type 2 diabetes on liver fat content, insulin clearance, and insulin action on glucose production and utilization, and on gene expression in adipose tissue.

**RESEARCH DESIGN AND METHODS**

This study was an investigator-initiated study not financially supported by manufacturers of metformin or rosiglitazone. A total of 20 diet-treated type 2 diabetic patients (13 women and 7 men, mean duration of diabetes 11 ± 2 months) participated in the study. If considered eligible after a screening visit, metabolic studies (body composition, liver fat, insulin sensitivity, glucose rate of disappearance [Ra], and glucose rate of disappearance [Rd], and adipose tissue biopsy for gene expression studies) were performed before and after 16 weeks of treatment with either rosiglitazone or metformin as detailed below. At the screening visit, the patients underwent a history and physical examination, and blood samples were taken for measurement of HbA1c, fasting plasma glucose, liver enzymes, thyroid-stimulating hormone, and GAD antibodies to exclude patients with late-onset type 1 diabetes. Other exclusion criteria included use of drugs, clinical evidence of cardiovascular or other diseases other than diabetes, abnormal serum creatinine, macroalbuminuria, proliferative retinopathy or maculopathy, excessive alcohol consumption (>20 g/day), or drug abuse. The patients were randomized using minimization of differences (for the variables listed below) between the treatment groups to receive, in a double-blind fashion, two identical-looking capsules twice a day (metformin group: metformin 1 g + placebo b.i.d., rosiglitazone group: rosiglitazone 4 mg + placebo b.i.d.) for 16 weeks. Of note, this method of randomization does not necessarily result in an exactly equal number of patients in two groups (25). The following variables were considered during randomization: age, sex, BMI, HbA1c, and duration of diabetes. At the follow-up visits (2, 4, and 12 weeks), blood pressure and weight were recorded, and blood samples were taken for measurement of liver enzymes, HbA1c, lipids, creatinine, and electrolytes, and adverse events were recorded. Baseline characteristics of the patients are shown in Table 1.

The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The experimental protocol was approved by the ethical committee of the Helsinki University Central Hospital.

**Intra-abdominal and subcutaneous fat, liver fat, and other measures of body composition.** A total of 16 T1-weighted transaxial scans reaching from 8 cm above to 8 cm below the umbilical line were taken by using a 1.5-Tesla (GE Signa, Milwaukee, WI) magnetic resonance scanner with a 10-cm-thick slice having a repetition time (TR) of 138.9 ms, echo time (TE) 4.1 ms, as previously described (24). The reproducibility of repeated measurements of subcutaneous and intra-abdominal fat as determined on two separate occasions in our laboratory in nondiabetic subjects are 3 and 5% (25). Liver fat was measured with proton spectroscopy as previously described (26). The reproducibility of repeated measurement of liver fat in nondiabetic subjects studied in our laboratory on two occasions is 11% (25). The percent body fat was determined by using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model #BIA-101A; RJL Systems, Detroit, MI). To calculate the waist-to-hip ratio, waist was measured midway between spinous iliaca superior and the lower rib margin and hip at the level of the greater trochanters.

**Insulin sensitivity.** The patients were admitted to the hospital on the evening before the study at 7:00 p.m. to determine rates of glucose production (Ra) and Rd, a primed-continuous intravenous infusion of [3-3H]glucose was started at 4:00 a.m. and continued for a total of 660 min, as previously described (27). Before starting the insulin infusion at 9:00 a.m., another catheter (Venflon; Viggo-Spectramed, Helsingborg, Sweden) was inserted in a dorsal hand vein retrogradely. The hand was kept in a heated (60°C) chamber for withdrawal of arterialized venous blood. Baseline blood samples were taken for measurement of fasting plasma glucose; glucose rate of disappearance (Ra), HbA1c; triglycerides; total, HDL, and LDL cholesterol; free fatty acids (FFAs); and serum free insulin concentrations. A fat aspiration biopsy from abdominal subcutaneous area was then taken under local anesthesia, as previously described (28). At 9:00 A.M., after a 300-min equilibrium period, a primed-continuous (0.3 mU·kg⁻¹·min⁻¹) infusion of insulin (Actrapid Human; Novo Nordisk, Bagsvaerd, Denmark) was started, as previously described (29). Because of the different glucose production rates in humans, the infusion rate is very sensitive to suppression by insulin (complete suppression in normal subjects at an insulin infusion rate of 0.5 mU·kg⁻¹·min⁻¹) (30). We chose a low insulin infusion rate to be able to accurately assess hepatic insulin sensitivity. Plasma glucose was adjusted to and then maintained at ~8 mmol/l (~144 mg/dl) for 360 min both before and after treatment. During the insulin infusion, blood samples for measurement of serum-free insulin and FFA concentrations and for glucose specific activity were withdrawn as previously described (27). Total RNA and complementary DNA preparation. Frozen tissue (50–150 mg) was homogenized in 2 ml RNA STAT-60 (Tel-Test, Friendswood, TX), and total RNA was isolated according to the manufacturer’s instructions, as previously described (31). After DNase treatment (RNase-free DNase set; Qiagen, Hilden, Germany), RNA was purified using the RNeasy mini kit (Qiagen). RNA concentrations were measured using the RiboGreen fluorescent nucleic acid stain (RNA quantitation kit; Molecular Probes, Eugene, OR). The quality of RNA was checked by agarose gel electrophoresis. Average yields of total RNA were 3 ± 1 µg per 100 mg adipose tissue wet weight and did not differ between the groups. Isolated RNA was stored at −80°C until the quantification of the target mRNA. A total of 0.1 µg RNA was transcribed into complementary DNA using M-MLV reverse transcriptase (Life Technologies, Paisley, U.K.) and oligo (dT)₁₂–₁₈ primer.

**Quantification of β-actin, PPAR-γ, lipoprotein lipase, and adiponectin.** Quantification of β-actin, PPAR-γ, lipoprotein lipase (LPL), and adiponectin mRNA concentrations were performed by RT-PCR using LightCycler technology (Roche Diagnostics, Mannheim, Germany), as recently described in detail (31). A standard curve for PPAR-γ was created using purified cloned plasmid cDNA (QIAquick PCR purification kit; Qiagen). For human β-actin, adiponectin, and LPL expressions, standard curves were created from a specific PCR product. To account for differences in RNA loading, PPAR-γ, adiponectin, and LPL were expressed relative to β-actin. The mRNA concentration of human β-actin was not different between the groups (258 ± 29 in the rosiglitazone group and 199 ± 25 in the metformin group, NS). [3-3H]glucose specific activity and calculation of glucose kinetics. Glucose specific activity, Ra and Rd were determined as previously described (27).

Because endogenous Ra is very sensitive to even small changes in serum insulin concentrations (30), hepatic insulin resistance was calculated by multiplying basal plasma insulin concentrations (32). The percent suppression of basal endogenous Rd during the last 2 h (540–660 min) by insulin was used as a measure of the sensitivity of endogenous glucose production to insulin (percent suppression of endogenous Rd).

**Other analytical procedures.** Serum adiponectin concentrations were measured using a commercial enzyme-linked immunosorbent assay (Human Adiponectin ELISA kit; B-Bridge International, San Jose, CA). Plasma glucose, serum-free insulin, HbA1c, FFAs, lipids, and alanine aminotransferase (ALT) concentrations were determined as previously described in detail (24).

### Table 1: Baseline characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Rosiglitazone</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>M/F</td>
<td>3/6</td>
<td>4/7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50 ± 3</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.6 ± 0.9</td>
<td>30.6 ± 1</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.97 ± 0.02</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>Serum fasting insulin (mU/l)</td>
<td>12.3 ± 1.4</td>
<td>13.9 ± 2.2</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>4.7 ± 0.4</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.40 (1.27, 1.73)</td>
<td>1.96 (1.29, 4.14)</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol/l)</td>
<td>2.95 ± 0.32</td>
<td>2.93 ± 0.18</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/l)</td>
<td>1.12 ± 0.06</td>
<td>1.13 ± 0.07</td>
</tr>
</tbody>
</table>

Data are means ± SE, except for serum triglycerides, which are shown as median (25th and 75th percentiles).
TABLE 2
Characteristics of the patients before and after treatment with rosiglitazone and metformin

<table>
<thead>
<tr>
<th></th>
<th>Rosiglitazone Before</th>
<th>Rosiglitazone After</th>
<th>Metformin Before</th>
<th>Metformin After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>8.8 ± 0.8</td>
<td>7.3 ± 0.4</td>
<td>8.2 ± 0.7</td>
<td>6.7 ± 0.2</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.0 ± 0.4</td>
<td>6.6 ± 0.3</td>
<td>6.9 ± 0.4</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>88.5 ± 4.6</td>
<td>89.1 ± 4.6</td>
<td>84.1 ± 4.0</td>
<td>81.7 ± 3.7</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>58.5 ± 3.3</td>
<td>58.6 ± 3.1</td>
<td>56.3 ± 3.1</td>
<td>55.6 ± 3.1</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>102 ± 3</td>
<td>99 ± 3</td>
<td>103 ± 3</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>Intra-abdominal fat volume (cm³)</td>
<td>1,925 ± 253</td>
<td>1,960 ± 279</td>
<td>2,167 ± 209</td>
<td>2,045 ± 269</td>
</tr>
<tr>
<td>Subcutaneous fat volume (cm³)</td>
<td>4,275 ± 567</td>
<td>4,472 ± 651</td>
<td>3,969 ± 518</td>
<td>3,786 ± 490</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05, †P < 0.01 for before vs. after treatment.

Statistical analysis. Paired and unpaired t tests were used to compare changes between rosiglitazone and metformin groups. Non-normally distributed variables were logarithmically transformed. Effects of group, group times time, and time (insulin) on serum FFA concentrations were analyzed using ANOVA for repeated measures using SysStat Statistical Package (SysStat version 10; SysStat, Evanston, IL). Pearson’s (for normally distributed variables) or Spearman’s rank (for non-normally distributed variables) correlation coefficients were used to calculate correlation coefficients between selected variables. All calculations were made by GraphPad Prism version 3.0 (GraphPad, San Diego, CA). Data are shown as means ± SE. P < 0.05 was considered statistically significant.

RESULTS

Glycemic control, lipids, body weight, and composition. At baseline, the rosiglitazone and metformin groups were comparable with respect to sex, age, BMI, waist-to-hip ratio, serum insulin, and lipid concentrations (Table 1). Fasting plasma glucose and HbA1c decreased similarly in both groups (Table 2). Serum triglycerides decreased by 0.23 ± 0.18 mmol/l in the rosiglitazone group (NS) and by 0.37 ± 0.18 mmol/l (P < 0.05 for 16 vs. 0 weeks) in the metformin group. Serum HDL cholesterol increased significantly in the rosiglitazone group (by 0.25 ± 0.1 mmol/l, P < 0.05) but not in the metformin group (0.09 ± 0.05 mmol/l, NS), and LDL cholesterol remained unchanged. Hemoglobin decreased by 5% (P < 0.01) but not in the metformin group (0.09 ± 0.05 mmol/l, NS), implying that insulin resistance created by the exogenous insulin infusion, serum insulin increased significantly less in the rosiglitazone group than in the metformin group after therapy (mean increase 19 ± 2 vs. 28 ± 3 mU/l, P < 0.02 for difference in increments between the groups) compared with before therapy (25 ± 2 vs. 27 ± 3 mU/l, NS), implying that insulin clearance had increased during rosiglitazone therapy. Insulin clearance increased from 16 ± 2 to 20 ± 1 ml · kg⁻¹ · min⁻¹ (P = 0.02) with rosiglitazone. Serum insulin concentrations during the insulin infusion were also lower after therapy with rosiglitazone than metformin (27 ± 1 vs. 37 ± 3 mU/l, P = 0.01), whereas there was no difference between rosiglitazone and metformin therapy by 4 ± 1 and 4 ± 2 mU/l, respectively (Fig. 1). During the square-wave of hyperinsulinemia created by the exogenous insulin infusion, serum insulin increased significantly less in the rosiglitazone group than in the metformin group after therapy (mean increase 19 ± 2 vs. 28 ± 3 mU/l, P < 0.02 for difference in increments between the groups) compared with before therapy (25 ± 2 vs. 27 ± 3 mU/l, NS), implying that insulin clearance had increased during rosiglitazone therapy. Insulin clearance increased from 16 ± 2 to 20 ± 1 ml · kg⁻¹ · min⁻¹ (P = 0.02) with rosiglitazone. Serum insulin concentrations during the insulin infusion were also lower after therapy with rosiglitazone than metformin (27 ± 1 vs. 37 ± 3 mU/l, P = 0.01), whereas there was no difference between rosiglitazone and metformin therapy by 4 ± 1 and 4 ± 2 mU/l, respectively (Fig. 1).
before therapy (37 ± 2 vs. 41 ± 4 mU/l, NS) (Fig. 2). Serum insulin concentrations were also lower after therapy than before therapy in the metformin group, but the difference was identical to that in the fasting insulin concentrations (4 ± 2 mU/l). This difference was significantly less than that observed with rosiglitazone (10 ± 2 mU/l, P < 0.01 vs. metformin).

During intravenously maintained hyperinsulinemia, steady-state plasma glucose concentrations (30–360 min) were similar in both groups before therapy (8.2 ± 0.3 vs. 8.2 ± 1.4 mmol/l, rosiglitazone vs. metformin, NS) and after therapy (7.8 ± 0.1 vs. 8.0 ± 0.2 mmol/l, respectively, NS) treatment. Basal glucose production was unchanged in both groups (rosiglitazone 2.1 ± 0.1 vs. 2.0 ± 0.1 mg·kg⁻¹·min⁻¹, NS; metformin 2.1 ± 0.1 vs. 2.1 ± 0.1 mg·kg⁻¹·min⁻¹, NS, 0 vs. 16 weeks). Because of the decrease in serum insulin concentrations, basal hepatic insulin resistance decreased significantly in both groups [rosiglitazone 26 ± 3 vs. 15 ± 3 (mg·kg⁻¹·min⁻¹) × (mU/l), P < 0.002; metformin 29 ± 6 vs. 20 ± 2 (mg·kg⁻¹·min⁻¹) × (mU/l), P < 0.05] (Fig. 1).

Before treatment, hepatic Rₘ during hyperinsulinemia (0.29 ± 0.2 vs. 0.37 ± 0.22 mg·kg⁻¹·min⁻¹, NS; rosiglitazone vs. metformin group) and the percent suppression of Rₘ by insulin (−89 ± 13 vs. −89 ± 13%, NS; rosiglitazone vs. metformin) were similar in both groups. After treatment, hepatic glucose production during hyperinsulinemia was lower after metformin (0.07 ± 0.16 vs. 0.56 ± 0.15 mg·kg⁻¹·min⁻¹, P = 0.04) than rosiglitazone. The percent suppression by insulin was also lower after metformin than rosiglitazone treatment (−97 ± 8 vs. −72%, P = 0.04, metformin vs. rosiglitazone) at the higher insulin concentrations (vide supra). Despite the lower increment in serum insulin concentrations in the rosiglitazone versus the metformin group after therapy, insulin-stimulated Rₘ decreased significantly in both groups (3.5 ± 0.3 vs. 3.5 ± 0.3 mg·kg⁻¹·min⁻¹, NS) (Fig. 3). When corrected for the difference in insulin concentrations (29), Rₘ increased by 70% in the rosiglitazone group from 0.10 ± 0.02 to 0.17 ± 0.02 (mg·kg⁻¹·min⁻¹)/(mU/l) and remained unchanged in the metformin group: 0.09 ± 0.01 vs. 0.10 ± 0.01 (mg·kg⁻¹·min⁻¹)/(mU/l) (0 vs. 16 weeks, NS).

Serum fasting FFAs decreased significantly and similarly with rosiglitazone (−17 ± 4%, from 649 ± 76 to 537 ± 69 mmol/l, P < 0.01) and metformin (−15 ± 6%, from 711 ± 62 to 599 ± 61 mmol/l, P < 0.02, 0 vs. 16 weeks). Serum FFAs during hyperinsulinemia were also lower after therapy in both the rosiglitazone (355 ± 39 vs. 277 ± 42 mmol/l, P = 0.005, 16 vs. 0 weeks) and metformin (396 ± 40 vs. 331 ± 39 mmol/l, P = 0.001) groups. The percent suppression of serum FFAs by insulin was similar both before and after rosiglitazone (55 ± 3 vs. 56 ± 2%, 0 vs. 16 weeks) and metformin (56 ± 2 vs. 59 ± 8%, respectively).

**Liver fat and serum ALT.** In the rosiglitazone group, liver fat decreased by 51 ± 7% from 15 ± 3 to 7 ± 2% (P = 0.003), whereas there was no change in the metformin group (13 ± 3 vs. 14 ± 3%, NS) (Fig. 4). Serum ALT decreased significantly in the rosiglitazone group from 42 ± 7 to 29 ± 3 IU/l (P < 0.05) but remained unchanged in the metformin group (52 ± 9 vs. 42 ± 6 IU/l, NS).

**Serum adiponectin concentrations and mRNA expressions of PPAR-γ, adiponectin, and LPL.** Serum adiponectin concentrations increased by 123% in the rosiglitazone group, from 5.6 ± 0.7 to 12.5 ± 1.8 mg/l (P < 0.001), but remained unchanged in the metformin group (6.1 ± 1.0 to 6.1 ± 1.0 mg/l, NS) (Fig. 4). The increase in serum adiponectin correlated with absolute and relative decreases in liver fat (Fig. 4). PPAR-γ, adiponectin, and LPL mRNA concentrations in subcutaneous adipose tissue increased significantly in the rosiglitazone group but not the metformin group (Fig. 5). Fat cell size of subcutaneous adipose tissue did not change with either rosiglitazone (69 ± 3 vs. 71 ± 3 μM, NS) or metformin (65 ± 4 vs. 65 ± 2 μM, NS, 0 vs. 16 weeks).
DISCUSSION

The present study was undertaken to examine the mechanism of action of rosiglitazone compared with metformin in humans during chronic treatment. The novel data include the following: 1) metformin increases hepatic insulin sensitivity without changing liver fat content; 2) rosiglitazone reduces liver fat content, and this is associated with an increase in insulin clearance; and 3) chronic treatment with rosiglitazone but not metformin increases PPAR-γ, LPL, and adiponectin expression in adipose tissue.

The effects of rosiglitazone and metformin on liver fat are in marked contrast to animal studies. We found metformin to sensitize the liver without changing hepatic fat content. The ability of metformin to increase hepatic insulin sensitivity has been documented in several previous human studies (3–5,33,34), but liver fat was not measured. In isolated hepatocytes and metformin-treated rats, lipogenesis is inhibited because of AMPK-induced inactivation of acetyl-CoA carboxylase and suppression of lipogenic enzyme and transcription factor expression (1). It was also suggested, although not proven by direct measurements (possibly because of the lack of liver fat in normal rats), that metformin decreases hepatic steatosis and might therefore be useful in treating subjects with insulin resistance and a fatty liver. Although the present data provide no information regarding the cellular mechanism of action of metformin, they demonstrate that the predicted consequence of inhibition of lipogenesis, a reduction in steatosis, does not characterize metformin action in humans. Although in the study of Zhou et al. (1) activation of AMPK (1.3-fold increase) was observed after incubation of hepatocytes for 39 h in the presence of therapeutic concentrations of metformin (10 μmol/l), the dose used to treat rats in vivo was 10-fold higher (286 mg/kg) than that in the present study (24 mg/kg). Metformin has been, at much higher doses than in the study of Zhou et al., shown to inhibit oxidative phosphorylation and lower cellular ATP levels (35). AMPK is very sensitive to even small changes in AMP and ATP levels. While ATP levels were reported not to change, no measurements of AMP were given in the study of Zhou et al. (1). In ob/ob mice with a fatty liver, metformin reverses hepatomegaly and steatosis (36). Metformin has also been suggested to decrease serum ALT levels in patients with nonalcoholic steatohepatitis, but this study was uncontrolled (2).

The present study is the first to document a decrease in liver fat in a double-blind randomized trial by PPAR-γ agonism and is keeping with previous data showing a similar decrease in liver fat in uncontrolled studies using either pioglitazone (8) or rosiglitazone (7,10,37). These data again contrast those in mice, in which treatment with PPAR-γ agonists appears to increase rather than decrease liver fat (38,39). In such studies, PPAR-γ expression has increased in the liver (40). Lack of PPAR-γ expression in the liver protects mice from developing hepatosteatosis (41). The normal human liver has very low PPAR-γ expression, but whether this applies to a fatty liver is unknown (42).

Several mechanisms could underlie the ability of PPAR-γ agonists to reduce liver fat content. First, it has been suggested that lowering of FFAs leads to “keeping FFAs where they belong, i.e., in adipose tissue” and a decrease in FFA availability for hepatic lipogenesis (10). In the present study, rosiglitazone and metformin had similar effects on FFA concentrations. These effects seem to be, based on measurement of in vivo rates of FFA turnover (6), due to inhibition of lipolysis. We have previously shown in a 6-month placebo-controlled study that rosiglitazone can decrease liver fat without changing adipose tissue mass in patients with HIV lipodystrophy (43). These considerations argue against FFA flux to the liver being the only regulator of liver fat content. In normal mice, rosiglitazone has no effects on hepatic mRNA levels and
acts exclusively via effects in adipose tissue (41). These effects include an increase in adiponectin expression, as was also observed in the present study in patients with normal or increased amounts of adipose tissue. Adiponectin increases hepatic insulin sensitivity in mice by activating fatty acid oxidation and inhibiting phosphoenolpyruvate carboxykinase expression (21,22). Consistent with the idea that adiponectin is important in mediating changes in liver fat content and possibly hepatic insulin sensitization, we found a significant correlation between changes in adiponectin and liver fat content (Fig. 4). Troglitazone treatment has previously been shown to increase adiponectin concentrations in human adipose tissue (44).

Approximately 50% of insulin is cleared by the liver in humans (45). Fat accumulation in the liver has been associated with impaired insulin clearance, when measured as in the present study (14). In vitro, hepatocytes loaded with triglycerides in vitro after incubation with physiological concentrations of FFA clear less insulin than normal hepatocytes (15). In the in situ perfused rat liver, hepatic triglycerides and insulin clearance are closely correlated \((r = -0.8-0.9)\) (46) and addition of FFAs (within the physiological range up to 1,000 \(\mu\text{mol/l}\)) reduces hepatic removal of insulin by 40% (47). This implies that fasting hyperinsulinemia associated with the fatty liver is at least partly due to impaired insulin clearance. Insulin clearance was increased and liver fat content decreased by rosiglitazone therapy. Because fat-free mass was unchanged and at least based on creatinine or urinary albumin excretion (data not shown), there were no changes in renal function, and the decrease in liver fat likely explains the improvement in insulin clearance. The commonly observed decrease in serum fasting insulin by rosiglitazone can therefore at least in part be attributed to an increase in insulin clearance. Because of the change in insulin clearance and lower insulin concentrations both in the basal state and during hyperinsulinemia, it is difficult to estimate whether true hepatic insulin sensitization occurred in the present study with rosiglitazone. Such evidence has to rely on previous studies (albeit uncontrolled), where higher insulin infusion rates were used (6,10). The reason(s) why rosiglitazone has not previously been noted to increase insulin clearance remains speculative. First, most studies did not quantitate hepatic fat content, and if hepatic fat was low at baseline, it may not have changed during therapy (6,10). In the study of Miyazaki et al. (6), serum free insulin concentrations during an insulin infusion of 1 \(\text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) were 12 \(\text{mU/l}\) lower after rosiglitazone than after placebo (61 vs. 73 \(\text{mU/l}\)), but this difference was not statistically significant. In this study, liver fat content was not determined, and the insulin infusion lasted 90 min, compared with 360 min in the present study. Mayerson et al. (10) reported a significant decrease (39%) in hepatic triglyceride content in the rosiglitazone group, but there were no differences in insulin levels during the low- or high-dose insulin clamp. In another study, rosiglitazone decreased liver fat content by 22%, but insulin levels during the insulin clamp were not reported (7). In the present study, we chose a long-lasting low-dose exogenous insulin infusion to maximize the likelihood of detecting differences in insulin clearance and hepatic insulin sensitivity. Clearly, metformin did improve hepatic insulin sensitivity consistent with previous studies (4) and in the absence of a change in liver fat content. Together, the data imply that a decrease in liver fat content increases insulin clearance but is not necessary for metformin-induced hepatic insulin sensitization in humans.

Troglitazone (3), pioglitazone (8), and rosiglitazone (48) have been shown to increase peripheral glucose uptake in previous studies. Whereas troglitazone was suggested to act primarily by increasing the rate of peripheral glucose disposal, both pioglitazone and rosiglitazone have been shown to increase peripheral and hepatic insulin sensitivity (6,8,48). In the present study, we confirmed the ability of rosiglitazone to enhance peripheral insulin sensitivity. Because metformin was as effective in improving glycemic control and lowering FFA concentrations, and it decreased body weight in contrast to rosiglitazone, this

**FIG. 5.** Effects of 16 weeks of rosiglitazone (■) and metformin (□) treatment on PPAR-γ, adiponectin, and LPL mRNA concentrations in subcutaneous adipose tissue expressed as relative to β-actin. *\(P < 0.05\) for before vs. after treatment.
increase cannot be attributed to changes in gluco-toxicity, lipotoxicity, or body weight. Recently, rosiglitazone treat-
ment in patients with type 2 diabetes was shown to improve downstream insulin signaling in human skeletal muscle by increasing insulin stimulation of insulin receptor substrate-1 tyrosine phosphorylation and p85 association with insulin receptor substrate-1 (49). Given the low PPAR-γ expression in skeletal muscle compared with adipose tissue, it is unclear whether these effects are direct or indirect.

In conclusion, in humans, metformin acts primarily by sensitizing the liver to insulin without changing its fat content. Rosiglitazone markedly reduces liver fat and increases insulin clearance. It doubles adiponectin concentra-
tions, which may contribute to the decrease in liver fat. Unlike metformin, rosiglitazone also increases peripheral insulin sensitivity.

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