Decreased whole body lipolysis as a mechanism of the lipid-lowering effect of pioglitazone in type 2 diabetic patients

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Gastaldelli A, Casolaro A, Ciociaro D, Frascerra S, Nannipieri M, Buzzigoli E, Ferrannini E. Decreased whole body lipolysis as a mechanism of the lipid-lowering effect of pioglitazone in type 2 diabetic patients. Am J Physiol Endocrinol Metab 297:E225–E230, 2009. First published May 5, 2009; doi:10.1152/ajpendo.90960.2008.—Pioglitazone has been shown to reduce fasting triglyceride levels. The mechanisms of this effect have not been fully elucidated, but decreased lipolysis may contribute to blunt the hypertriglyceridemic response to a meal. To test this hypothesis, we studied 27 type 2 diabetes mellitus (T2DM) patients and 7 sex-, age-, and body mass index-matched nondiabetic controls. Patients were randomized to pioglitazone (45 mg/day) or placebo for 16 wk. Whole body lipolysis was measured as the [1H5]glycerol rate of appearance (Ra) in the fasting state and for 6 h following a mixed meal. Compared with controls, T2DM had higher postprandial profiles of plasma triglycerides, free fatty acid (FFA), and β-hydroxybutyrate, and a decreased suppression of glycerol Ra (P < 0.04) despite higher insulin levels [268 (156) vs. 190 (123) pmol/l, median (interquartile range)]. Following pioglitazone, triglycerides and FFA were reduced (P = 0.05 and P < 0.04, respectively), and glycerol Ra was more suppressed [−40 (137) vs. +7 (202) μmol/min of placebo, P < 0.05] despite a greater fall in insulin [−85 (176) vs. −20 (58) pmol/l, P = 0.05]. We conclude that, in well-controlled T2DM patients, whole body lipolysis is insulin resistant, and pioglitazone improves the insulin sensitivity of lipolysis.

Patients with type 2 diabetes (T2DM), especially if obese, are characterized by high fasting and postprandial triglyceride levels (27) and day-long elevations in plasma free fatty acid (FFA) concentrations (23). This form of dyslipidemia, which is usually coupled with low high-density lipoprotein-cholesterol concentrations and a predominance of small, dense low-density lipoprotein-cholesterol particles, is atherogenic (21) and contributes to the cardiovascular risk of T2DM, especially in women (3). Diabetic hypertriglyceridemia is initiated by the export of large triglyceride-rich lipoproteins released from expanded intrahepatic fat depots (1, 26). Insulin is a potent suppressor of lipolysis and restrains the release of FFA from the adipocyte and skeletal muscle tissue by inhibiting hormone-sensitive lipase. In T2DM, the ability of insulin to lower plasma FFA concentrations is reduced (12). Therefore, the use of pharmacological agents that enhance insulin sensitivity of glucose metabolism, such as the thiazolidinediones (TZD), should also improve dyslipidemia.

As reviewed by Betteridge (5), in clinical trials, pioglitazone has been consistently shown to improve, along with glycemic control, the lipid profile of T2DM patients when used in monotherapy or in combination with other oral antidiabetic agents. Whether this effect is directly related to sensitization of lipolysis in T2DM has not been determined. We therefore studied whole body lipolysis, as estimated from the rate of systemic appearance of labeled glycerol, in the fasting state and following a mixed meal in a group of T2DM patients randomized to a 16-wk treatment with pioglitazone or placebo.

METHODS

Study design. We recruited 38 T2DM patients and 7 age- and weight-matched nondiabetic control subjects. Patients who were previously treated with insulin or TZDs were excluded. All subjects were in good general health; controls were not taking any medication known to affect glucose tolerance, and T2DM patients who were taking sulfonylureas or metformin discontinued the medication 6 wk before study. After the baseline studies, T2DM patients were randomized in a double-blind fashion to receive pioglitazone (45 mg/day at breakfast, the maximum of the recommended dose range of 15–45 mg/day) or placebo (in a 2:1 ratio) for 16 wk. All patients were instructed to follow a standard isocaloric diet and to return to our clinic every 2 wk for followup visits. Five patients were not eligible for randomization, one patient (randomized to placebo) dropped out because of unsatisfactory glycemic control (i.e., fasting plasma glucose >15 mmol/l on 2 consecutive occasions within 1 wk) and five patients (1 in the placebo and 4 in the pioglitazone group) dropped out for reasons unrelated to the protocol. Data are therefore presented for the 27 patients who completed the study. The study protocol was approved by the Institutional Review Board, and written consent was obtained from each patient before participation. Data on glucose turnover have been published previously (10).

Study protocol. Subjects were admitted to our Clinical Research Unit after an ~12-h overnight fast. A cannula was inserted in an antecubital vein for infusion of test substances. Another catheter was inserted retrogradely in an ipsilateral wrist vein for blood sampling, and the hand was kept in a heated box at 65°C. A primed (1.5 μmol/kg) continuous (0.10 μmol·min⁻¹·kg⁻¹) infusion of [1H5]glycerol (Cambridge Isotope Laboratories, Boston, MA) was initiated and continued until the end of the study. During the last 20 min of the basal equilibration period (40–60 min), plasma samples were taken at 10-min intervals for the determination of blood glycerol concentrations and [1H5]glycerol enrichment. After the equilibration period, a standard mixed meal (75 g glucose as an aqueous solution, 50 g parmesan cheese, and one 50-g egg for a total of 585 kcal, of which 18% protein, 31% fat, and 51% carbohydrate) was administered, and blood samples for the determination of glycerol concentration and enrichment, and hormone and substrate (glucose, lactate,
pyruvate, alanine, β-hydroxybutyrate) levels were obtained 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min thereafter.

Analytical methods. Plasma glucose was determined by the glucose oxidase method (Beckman Glucose Analyzer, Fullerton, CA), and plasma insulin was determined by radioimmunoassay (Linco Research, St. Charles, MO). Hemoglobin A1c concentration was measured by affinity chromatography (Biochemical Methodology, Akron, OH), and plasma FFA was measured spectrophotometrically (Wako Chemicals, Neuss, Germany). [3H]glycerol enrichment was measured by gas chromatography/mass spectrometry (GC/MS) as previously described (31). Briefly, after deproteinization, plasma samples were derivatized with pyridine and acetic anhydride (1:1) and then reconstituted with ethyl acetate (80 μl). Samples were then injected (2 μl) in the GC/MS (Agilent 5890/5972; Palo Alto, CA), and enrichments were evaluated as the peak area ratio between fragments of mass 148 and 145, after correction for baseline values. Whole blood glycerol, lactate, pyruvate, and β-hydroxybutyrate concentrations were determined spectrophotometrically on an Beckman Syncron CX7 analyzer (Global Medical Instrumentation, Ramsey, MN). For these assays, blood samples were collected in iced tubes containing 1 M perchloric acid for immediate deproteinization; the supernatant obtained from centrifugation was stored at −20°C and assayed within 30 days.

Data analysis. Fat-free mass (FFM) was measured by electrical bioimpedance; fat mass (FM) was obtained as the difference between body weight and FFM. During the last 20 min of the tracer equilibration period, both plasma glycerol concentrations and [3H]glycerol enrichments were stable in all subjects. Therefore, the rate of appearance (Ra) of endogenous glycerol was calculated as the ratio of the tracer infusion rate to the plasma tracer enrichment (mean of 3 determinations). After meal ingestion, glycerol Ra was calculated from [3H]glycerol enrichment using Steele’s equation (11).

Statistical analysis. Data are presented as means ± SE or, when nonnormally distributed, as the median (interquartile range). Group comparisons were carried out by ANOVA, χ², or Mann-Whitney U-test, for continuous, nominal, and nonnormally distributed continuous variables, respectively. Changes in time course by group (controls vs. T2DM) were tested by two-way ANOVA for repeated measures over time. The effect of treatment (pioglitazone or placebo) was tested by ANOVA for doubly repeated measures (time and treatment). Regression analysis was used to explore associations between continuous variables. Differences between regression lines were tested with the use of a mixed model including group as a factor (controls or T2DM) and an interaction term between group and the independent variable.

RESULTS

The diabetic patients were matched to the control subjects by sex, age, and adiposity. Plasma glucose and insulin concentrations were higher in diabetic than controls both under fasting conditions and during the 6-h postprandial period (Table 1). Circulating concentrations of FFA and glycerol decreased significantly (P < 0.0001 for both), and plasma triglycerides rose (P < 0.0001), during meal absorption. The plasma FFA profile was significantly higher (P = 0.01), and the plasma triglyceride profile tended to be higher (P = 0.07), in patients than in controls, whereas the blood glycerol profile did not differ significantly (Fig. 1). Blood lactate and pyruvate concentrations rose at 60 min postmeal and declined thereafter in both groups (P < 0.0001 for both); their time course did not differ between groups (data not shown). Blood β-hydroxybutyrate levels were significantly (P < 0.0001) suppressed during the meal but were higher in patients than controls throughout the meal (P = 0.04) (data not shown).

In patients vs. controls, glycerol Ra tended to be higher in the fasting state (P = 0.1); after the meal, it decreased in a similar time course as plasma FFA in both groups (P < 0.0001), but remained significantly (P < 0.04) higher in patients than controls, especially during the first ~2.5 h postmeal (Fig. 2). Glycerol Ra was reciprocally related to plasma insulin concentrations throughout the meal; the slope of such relationship was significantly (P < 0.04) flatter in patients than controls, i.e., glycerol Ra was higher in diabetic than nondiabetic subjects at each plasma insulin concentration (Fig. 2).

After the baseline measurements, the diabetic patients were randomized to pioglitazone (n = 17) or placebo (n = 10). The patients in the two treatment arms were matched for age [61 ± 2 vs. 62 ± 2 yr, P = not significant (NS), pioglitazone vs. placebo], body weight (78.4 ± 2.3 vs. 76.9 ± 3.8 kg, P = NS), and body mass index (28.2 ± 0.8 vs. 29.7 ± 0.8 kg/m, P = NS). Compared with placebo, pioglitazone treatment was associated with a significant drop in plasma glucose, insulin, and FFA levels both in the fasting state and postprandially (Table 2). In the placebo group, the responses of plasma FFA, triglycerides, and glycerol were not significantly different before and after treatment. In the pioglitazone group, both the FFA and triglyceride response were lower at 16 wk than at baseline, whereas the blood glycerol response was not significantly different (Fig. 3). None of the other metabolites (lactate, pyruvate, β-hydroxybutyrate) showed significant changes with treatment in either the placebo or the pioglitazone group (data not shown).

With pioglitazone, fasting glycerol Ra was decreased [and no longer significantly different from the value in controls, 153 (74) vs. 149 (39) µmol/min, P = NS] (Table 2) and better suppressed during the postmeal period (P = 0.05) compared with placebo, the average postmeal difference amounting to −40 (137) µmol/min vs. +7 (202) of placebo (Fig. 4). The slope of the reciprocal relationship between glycerol Ra and plasma insulin was no different before and after treatment in

Table 1. Clinical and metabolic characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>T2DM</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>4/3</td>
<td>11/16</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
<td>62±1</td>
<td>61±1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.0±0.6</td>
<td>28.7±0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74±1.1</td>
<td>78±2</td>
<td>NS</td>
</tr>
<tr>
<td>FMF, kg</td>
<td>51±2</td>
<td>54±1</td>
<td>NS</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>18±2</td>
<td>21±1</td>
<td>NS</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.3±0.2</td>
<td>7.0±0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>204±11</td>
<td>213±7</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>64±2</td>
<td>55±3</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>85 (68)</td>
<td>128 (75)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE or median with interquartile ranges in parentheses. F, females; M, males; BMI, body mass index; FFM, fat-free mass; Hb A1c, hemoglobin A1c; HDL, high-density lipoprotein. *For the difference between type 2 diabetes mellitus (T2DM) and controls; 1P ≤ 0.05 vs. fasting; NS, not significant.
DISCUSSION

The T2DM patients in the present study had similar FM as the nondiabetic subjects (controls, n = 7) and patients with type 2 diabetes (T2DM, n = 27). Data are given as the means ± SE. P values refer to the significance of the difference between the two curves (i.e., the interaction term by 2-way ANOVA for repeated measures). NS, not significant.

Fig. 1. Time course of plasma free fatty acid (FFA), plasma triglycerides, and blood glycerol concentrations during a mixed meal in nondiabetic subjects (controls, n = 7) and patients with type 2 diabetes (T2DM, n = 27). Data are given as the means ± SE. P values refer to the significance of the difference between the two curves (i.e., the interaction term by 2-way ANOVA for repeated measures). NS, not significant.

Table 2. Changes in metabolic variables after pioglitazone or placebo treatment

<table>
<thead>
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<th>Pioglitazone</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Body wt, kg</td>
<td>+1.2 ± 0.6</td>
<td>+0.1 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>−0.4 ± 0.27</td>
<td>+0.14 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>−1.0 ± 0.4</td>
<td>+0.8 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>−25 (54)</td>
<td>−3 (24)</td>
<td>0.04</td>
</tr>
<tr>
<td>Plasma FFA, μmol/l</td>
<td>−120 ± 84</td>
<td>+38 ± 48</td>
<td>0.07</td>
</tr>
<tr>
<td>Lipolysis, μmol/min</td>
<td>−83 (154)</td>
<td>+23 (226)</td>
<td>0.02</td>
</tr>
<tr>
<td>Mixed meal (0–360 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>−1.6 ± 0.3</td>
<td>0.0 ± 0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>−85 (176)</td>
<td>−20 (58)</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma FFA, mmol/l</td>
<td>−76 ± 48</td>
<td>+7 ± 22</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE or median with interquartile ranges in parentheses; n, no. of subjects. FFA, free fatty acid.

the placebo group, whereas it tended to be steeper after treatment in the pioglitazone group (P = 0.1).

DISCUSSION

The T2DM patients in the present study had similar FM as the nondiabetic subjects, were in fair metabolic control, and were only mildly dyslipidemic (only 9 patients had a fasting triglyceride level ≥150 mg/dl). However, in response to the meal, triglycerides tended to rise higher in patients than controls [85 (176) vs. 20 (58) mg/dl, P = 0.05]. Glycerol Ra was higher than in controls through the meal in the face of higher circulating insulin concentrations (Fig. 2). With regard to the relation of glycerol Ra to whole body lipolysis, ideally complete hydrolysis of a triglyceride molecule should give rise to a 3:1 ratio between FFA and glycerol Ra values. When measured in humans, the ratio of FFA Ra to glycerol Ra has been reported to average 2.6 following an overnight fast (14). The main sources of glycerol Ra are adipose tissue triglycerides and circulating lipoprotein triglycerides (via capillary endothelial lipoprotein-lipase). During insulin stimulation, the ratio of FFA to glycerol Ra falls significantly below three
(averaging 1.5) because with ongoing circulating lipoprotein triglyceride lipolysis glycerol is released in the systemic blood while FFA are efficiently taken up in tissues. For this reason, glycerol $R_a$ is a better approximation of whole body lipolysis under fasting conditions than in the insulinized state (14).

Thus, to the extent that glycerol $R_a$ reflects lipolysis (14), the current findings clearly document the presence of insulin resistance of lipolysis in the T2DM patients. Accordingly, the profile of plasma FFA also was shifted upward in patients vs. controls (Fig. 1). At least in part, the fate of this excess plasma FFA in the patients was hepatic oxidation because blood $\beta$-hydroxybutyrate concentrations were higher than in controls throughout the meal. Although indirect calorimetry was not performed in these patients, previous studies have documented higher rates of whole body fat oxidation in T2DM under insulin-stimulated conditions (9), reflecting metabolic inflexibility of insulin target tissues (15). Indeed, a reduced inhibitory effect of insulin on whole body net lipid oxidation has been shown to predict progression to diabetes in Pima Indians (17). It is therefore plausible that, in our patients, inappropriate lipolysis resulted in an increased reliance on fatty substrate oxidation for energy production. In turn, a higher degree of fat utilization likely contributed to the insulin resistance of glucose uptake [at the level of the liver (4) as well as the peripheral tissues (10)]. Recent studies have identified insulin suppressibility of circulating fatty acids, a readout of increased fat oxidation, as a major in vivo determinant of skeletal muscle insulin sensitivity (16).
the plasma FFA and triglyceride response to the meal were significantly reduced (Fig. 3). The mechanisms of these drug effects may be multiple. First, differentiation of preadipocytes into small, insulin-sensitive adipocytes would expand the adipose reservoir and clear circulating FFA more efficiently (32). Second, there is evidence that rosiglitazone increases blood flow to subcutaneous adipose tissue in diabetic patients (25, 29); pioglitazone may have a similar effect. Third, the postprandial activity of hormone-sensitive lipase may be reduced as a result of insulin sensitization (2). Fourth, expansion of lipoprotein lipase mass, and reduction of its inhibitor, ApoCIII, would increase catabolism of VLDL triglycerides (at least under fasting conditions) (19). TZDs may induce adipocyte glycerol kinase gene expression, thereby stimulating glycerol incorporation into triglyceride and reducing FFA release from the adipocyte (13), and rosiglitazone has been shown to decrease glycerol release from adipocytes at least during a low-dose insulin clamp (18). However, more recent studies by Cadoulal et al. (7) in human adipose tissue explants have shown no change in glycerol kinase activity following rosiglitazone incubation. In addition, Bogacka et al. (6) and Tan et al. (24) could not detect any significant induction of glycerol kinase in T2DM patients treated with TZDs. Finally, in isolated adipocytes of diabetic patients, rosiglitazone has been shown to upregulate glyceroneogenesis through phosphoenolpyruvate carboxykinase (7). One may presume that similar molecular events may follow chronic application of pioglitazone.

One unresolved issue is the basis for the difference between the lipid effects of pioglitazone and those of rosiglitazone in T2DM patients. As reviewed previously (5), clinical evidence consistently indicates that pioglitazone treatment is associated with decrements in triglycerides, fasting as well as postprandial. In contrast, rosiglitazone does not change fasting triglycerides (18) but improves postprandial triglyceride metabolism (25, 28). Some of these differences have been attributed to a peroxisome proliferator-activated receptor (PPAR)-α action of pioglitazone (22). It is worth noting that, in our patients receiving pioglitazone, we did not detect any significant induction of glycerol kinase in T2DM patients. As reviewed previously (5), clinical evidence consistently indicates that pioglitazone treatment is associated with decrements in triglycerides, fasting as well as postprandial. In contrast, rosiglitazone does not change fasting triglycerides (18) but improves postprandial triglyceride metabolism (25, 28). Some of these differences have been attributed to a peroxisome proliferator-activated receptor (PPAR)-α action of pioglitazone (22). It is worth noting that, in our patients receiving pioglitazone, we did not detect any significant induction of glycerol kinase in T2DM patients.

In summary, in well-controlled, mildly dyslipidemic T2DM patients, whole body lipolysis is insulin resistant, and pioglitazone restores insulin sensitivity of lipolysis, thereby contributing to improve the dyslipidemia. However, a direct link between improved adipose tissue insulin resistance and blood lipid profile could not be made and needs further study.

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GRANTS

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