Effects of Troglitazone in Young First-Degree Relatives of Patients With Type 2 Diabetes

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OBJECTIVE — Insulin resistance is a key characteristic of first-degree relatives of patients with type 2 diabetes. We therefore treated young, glucose-tolerant relatives with the insulin action enhancer troglitazone in order to determine the effects on insulin sensitivity, glucose metabolism, and glycogen synthase activity.

RESEARCH DESIGN AND METHODS — Relatives were randomized in a double-blind manner and treated for 12 weeks with either 200 mg troglitazone or placebo. Before and after treatment, an oral glucose tolerance test (OGTT) and a euglycemic-hyperinsulinemic clamp (40 mU·m⁻²·min⁻¹) were performed, including 3-¹H glucose infusion, glycolytic flux calculations, indirect calorimetry, and muscle biopsies.

RESULTS — Twelve relatives received troglitazone and 12 placebo (aged 30.8 ± 1.6 years, BMI 29.6 ± 0.8 vs. 30.5 ± 1.3 kg/m²; means ± SE). Area under the curve (AUC) for plasma glucose at the second OGTT was unchanged after troglitazone. In contrast, troglitazone reduced fasting (from 70.3 ± 6.9 to 52.2 ± 5.8 vs. 73.6 ± 11.0 to 73.3 ± 6.5 pmol/l, P < 0.02) and AUC plasma insulin (mean [CI] from 335.7 [230.9–488.1] to 277.4 [179.4–428.8] vs. 313.8 [218.2–451.2] to 353.9 [208.3–601.3] pmol/l, P < 0.05). Additionally, fasting plasma triglycerides were reduced by troglitazone (from 1.86 ± 0.33 to 1.38 ± 0.27 vs. 2.22 ± 0.44 to 2.35 ± 0.46 mmol/l, P < 0.01). Insulin-stimulated glucose disposal increased in the troglitazone group (from 208.3 ± 23.7 to 263.5 ± 30.4 vs. 197.1 ± 20.0 to 200.8 ± 20.8 mg·m⁻²·min⁻¹, P < 0.02) mainly due to increased glucose storage (from 99.9 ± 17.9 to 146.0 ± 25.3 vs. 87.1 ± 16.7 to 87.9 ± 15.7 mg·m⁻²·min⁻¹, P < 0.02), which took place without altering insulin-stimulated glycogen synthase activity.

CONCLUSIONS — In glucose-tolerant first-degree relatives, treatment with troglitazone improved insulin sensitivity almost 50%, primarily due to increased glucose storage. It is suggested that the use of insulin action enhancers can be especially valuable in this group of subjects with a known high risk for developing type 2 diabetes.

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The pathophysiology underlying type 2 diabetes is complex and believed to involve both genetically programmed factors and environmental influences, such as high-fat diets and sedentary lifestyles (1,2). Irrespective of the etiologic process leading to type 2 diabetes, the phenotypically most common form is characterized by a marked reduction in insulin-stimulated glucose uptake, predominantly in skeletal muscle, and a relative insulin deficiency (1,2). Insulin resistance has also been demonstrated in healthy first-degree relatives of type 2 diabetic patients and has therefore been proposed as an early marker of abnormal glucose metabolism (3,4). Moreover, premature atherosclerosis has been linked to insulin resistance; therefore treatments that improve insulin action seem pivotal in order to prevent or postpone the disease process.

Troglitazone, now superseded by rosiglitazone and pioglitazone, was the first of a new class of antidiabetic drugs, the thiazolidinediones (also known as glitazones), with insulin-sensitizing actions. The molecular target of these agents is thought to include the nuclear transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), which regulates numerous genes involved in adipocyte differentiation and lipid and glucose metabolism (5). PPARγ is mainly expressed in adipose tissue and, to a lesser extent, in muscle (6). The effects of PPARγ agonists on insulin sensitivity in skeletal muscle could therefore be indirect, involving adipose tissue PPARγ stimulation and leading to reductions in circulating and intramyocellular lipid levels (7–11) or alterations in the secretion of factors that modulate skeletal muscle insulin action (12–14). Alternatively, PPARγ agonists exhibit local (direct) intramyocellular actions, as suggested in some studies (10,15,16), although it remains to be investigated whether PPARγ agonists regulate gene expression in muscle by direct receptor activation.

Several clinical studies have suggested that thiazolidinediones may improve glycemic control, insulin sensitivity, and circulating lipid levels in type 2 diabetic patients (17–19). Troglitazone has also been shown to increase insulin sensitivity in subjects with impaired glucose tolerance (IGT) (20–22) and women with polycystic ovary syndrome (23). Furthermore, it was suggested in a longitudinal study (24) on Hispanic women with previous gestational diabetes that troglitazone may be able to protect from diabetes,
especially in women responding with large reductions in insulin output after taking the drug.

First-degree relatives of patients with type 2 diabetes are another high-risk population for the development of diabetes and therefore attractive for interventional means studies. Recently, we addressed the effects of troglitazone on the proximal steps of insulin signaling in skeletal muscle in a subgroup of our first-degree relatives and demonstrated that increased protein kinase B phosphorylation could be one of the mechanisms by which troglitazone improves insulin sensitivity (25). In the present placebo-controlled study, we have extended our studies on thiazolidinedione action in relatives. Thus, by using euglycemic-hyperinsulinemic clamps with tritiated glucose infusion, glycolytic flux calculations, indirect calorimetry, and skeletal muscle glycogen synthase activities, we sought to evaluate in detail the effect of troglitazone on the various glucose metabolic pathways. To minimize treatment-induced changes in metabolic control, which by themselves could have influenced the investigated parameters, only glucose-tolerant relatives were included.

RESEARCH DESIGN AND METHODS — First-degree relatives were recruited from the outpatient clinic at the Department of Endocrinology, Odense University Hospital, by questioning patients with verified type 2 diabetes. The relatives were overweight and all had at least one first-degree (parent) and one second-degree relative with type 2 diabetes. Other main criteria for their inclusion into the study were age 18–40 years, Caucasian ethnicity, no history of drug allergy, and no sign of endocrine/metabolic, cardiovascular, or liver disease as evaluated by medical history, clinical examination, and standard laboratory tests. Women were only included if they used adequate contraceptive measures (oral contraceptive medication or an intrauterine contraceptive device) throughout the study. Otherwise, no medication was allowed. Subjects were instructed not to change their diet and level of physical activity during the study and to avoid excessive physical exercise at least 3 days before metabolic investigations. Written informed consent was obtained from all subjects after the purpose and potential risks of the study were explained. The study was approved by the regional ethical committee and was in accordance with the Helsinki declaration.

All studies began at 8:00 A.M. after an overnight 12-h fast. Initially, a 3-h oral glucose tolerance test (OGTT) was performed to obtain metabolic profiles of glucose and insulin and to ensure normal glucose tolerance. Within the same week, a euglycemic-hyperinsulinemic clamp was conducted. Thereafter, the relatives were randomized in a double-blind manner to 12 weeks’ treatment with either 200 mg troglitazone or placebo once daily. At the end of treatment, the OGTT and clamp study were repeated. In addition, subjects were evaluated three times during the treatment period for possible adverse events and for collection of safety blood samples. In each woman, the OGTT and clamp measurements were conducted in the same phase of the menstrual cycle.

OGTT
Subjects drank 75 g glucose diluted in 300 ml water over a 2-min period. Venous blood samples were taken before the glucose load was administered, every 5 min during the first 20 min, at 10-min intervals until 2 h, and finally every 20 min during the last hour. In total, there were 18 samples.

Euglycemic-hyperinsulinemic clamp
A polyethylene catheter was inserted into an antecubital vein for infusion of 3-3H glucose, insulin, and glucose. Another catheter was inserted into a dorsal wrist vein of the opposite arm for blood sampling. During the study, the subjects remained in a supine position with the hand designated for collection of blood samples placed and maintained in a heated plexiglass box for arterialization of venous blood (26). After a 30-min relaxation period, a surface-adjusted primed constant 3-3H glucose infusion was given (DuPont-New England Nuclear, Boston, MA) at time zero (27). After a 120-min basal tracer equilibration period, insulin (Actrapid; Novo-Nordisk, Bagsvaerd, Denmark) was infused at a rate of 40 mU · m⁻² · min⁻¹ for 180 min and euglycemia maintained using a variable infusion of 18% glucose. To maintain plasma specific activity constant at the basal level during the clamp, 3-3H glucose was added to the glucose infusion, as previously described (27). Steady-state periods were defined as the last 30 min during basal and insulin-stimulated measurements, respectively.

Glucose and lipid oxidation
Indirect calorimetry was performed using a computerized flow-through canopy gas analyzer system (Datex; Datex, Helsinki, Finland), as previously described (4). After an equilibration period of 10 min, the average gas exchanges recorded over the two 30-min steady-state periods were used to calculate rates of glucose oxidation, lipid oxidation, and total energy expenditure (28). The protein oxidation rate was estimated from urinary urea nitrogen excretion (1 g nitrogen = 6.25 g protein) and corrected for changes in pool size (29).

Calculations
Insulin-stimulated glucose uptake (M) was defined as the glucose infusion rate during steady state. Rates of total glucose appearance (Rₚ) and glucose disposal (R₈) were calculated from the plasma concentrations of tritiated glucose and plasma glucose using Steele’s non–steady-state equations (27,30). In these calculations, the distribution volume of glucose was taken as 200 ml/kg body wt and the pool fraction as 0.65. Plasma glucose specific activities were maintained almost unchanged during basal and insulin stimulation. Thus, the calculated coefficients of variation of the specific activity values before and after treatment were 8.3 ± 0.8 and 5.7 ± 0.5% in the troglitazone group and 10.0 ± 1.1 and 7.2 ± 0.7% in the placebo group. Endogenous glucose production was calculated as the difference between Rₚ and the glucose infusion rate. The in vivo glycolytic flux rates were calculated from the rates of generation of plasma 3H₂O from 3-3H glucose. Briefly, this measurement is based on the knowledge that essentially all tritium in the C-3 position is lost to water at the level of the trioseisomerase reaction during the glycolytic process. Thus, the glycolytic flux rate can be calculated by multiplying the slope of the regression line obtained from the plot between time and 3H₂O content (in counts per minute per milliliter per minute) with total body water (in milliliters) estimated from the bioimpedance measurement and divided by plasma specific activity (in counts per minute per milligram) (31). In these calculations, plasma water was assumed to be 93% of
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the total plasma volume (31). Linearity (before/after) of the accumulation of plasma-irradiated water radioactivity was obtained from $-100$ to 0 min during basal measurements ($r = 0.95 \pm 0.01/0.95 \pm 0.01$ and $0.93 \pm 0.01/0.95 \pm 0.01$) and from 40 to 180 min during insulin infusion ($r = 0.97 \pm 0.01/0.96 \pm 0.01$ and $0.97 \pm 0.01/0.96 \pm 0.01$) in the troglitzone and placebo groups, respectively.

Rates of glucose storage were calculated as the difference between $R_d$ and glycolytic flux. Nonoxidative glucose disposal was calculated as the difference between $R_d$ and glucose oxidation as determined by indirect calorimetry.

All data of glucose metabolism are given as milligrams per meters squared per minute.

Body composition and total body water were estimated with the bioimpedance method using the formula of Kushner and Schoeller (32).

Disposition indexes were calculated in order to estimate the insulin secretion capacity in relation to insulin sensitivity, acknowledging the inverse hyperbolic relationship between insulin secretion and insulin action (33). Thus, the disposition index was calculated as $(\text{secretion}_\text{OGTT} \times M)$. In this index, first-phase insulin release during OGTT was calculated using the formula of Stumvoll et al. (34).

**Muscle biopsy**

At the end of each steady-state period a percutaneous skeletal muscle biopsy was taken under local anesthesia (2% lidocaine without epinephrine) from m. vastus lateralis 20 cm above the knee using the Bergstrom needle with suction (35). The biopsies were immediately frozen and stored in liquid nitrogen for later analysis.

**Glycogen synthase activity**

Before the biochemical analysis, the muscle biopsies were freeze-dried and dissected free of visible connective tissue, fat, and blood. Extraction of muscle samples and assays for glycogen synthase were performed as previously described (36) by a modification of the method of Thomas et al. (37). Briefly, glycogen synthase activity was measured by incubation of homogenates with $^{14}$C-uridine diphosphate glucose (UDPG) in the presence of near-physiological (0.1 mmol/l) and saturating (10 mmol/l) glucose-6-phosphate (G6P) concentrations. Glycogen synthase activity was expressed as nanomoles of UDPG incorporated into glycogen per min per milligram extract protein. The total concentration of UDPG ($^{14}$C-UDPG + cold UDPG) in the reaction mixture was 0.31 mmol/l. Fractional velocities (FVs) of glycogen synthase were calculated as the ratio between glycogen synthase activities assayed at 0.1 and 10 mmol/l G6P ($FV_{0.1}$).

**Analytical procedures.** Bedside plasma glucose (for adjustment of the variable glucose infusion during the clamp) was measured using a glucose oxidase method (Glucose Analyzer II; Beckmann Instruments, Fullerton, CA). Tritiated glucose activity was measured as previously described (27). Plasma glucose was measured using a glucose dehydrogenase method (Merck Diagnostica, Darmstadt, Germany) on an Axon-Technicon (Bayer Diagnostics, München, Germany). Plasma insulin and C-peptide were analyzed by a two-site, time-resolved immunofluorometric assay (38). Free fatty acids were measured by an enzymatic colorimetric method (Wako Chemicals, Neuß, Germany), as was triglyceride (Boehringer Mannheim Diagnostica, Mannheim, Germany). Serum urea nitrogen was measured using an enzymatic method on an Technicon Axon (Technicon Instruments, Tarrytown, NY). Uinary urea nitrogen was measured using an enzymatic method on a Cobas Mira S (Roche, Switzerland).

**Statistical analysis**

Differences in means within groups or between the groups were tested using paired or unpaired t tests, respectively (two tailed). To correct for baseline differences, the between-group analyses were carried out by comparing the incremental changes (baseline through week 12). In the absence of normal distribution, variables were logarithmically transformed to achieve normal distribution before statistical calculation. Area under the curve (AUC) during OGTT and basal clamp assessments were calculated as weighted means using trapezoidal integration. All analyses were performed with SPSS computer program version 7.5 for Windows (SPSS, Chicago, IL). Data are presented as means ± SE or means (95% CI), representing back-transformation of the logarithmic values. P values <0.05 were considered significant.

**RESULTS** — Twelve relatives received troglitazone and 12 placebo. The groups were comparable with respect to age and BMI (aged 30.8 ± 2.0 vs. 30.3 ± 1.6 years, BMI 29.6 ± 0.8 vs. 30.5 ± 1.3 kg/m²). The number of men was higher in the placebo group (nine versus six), which resulted in a higher mean body weight in this group. Of the women, four in the troglitzone and two in the placebo group were on contraceptive medication. During the study, there were no significant changes in weight (from 87.1 ± 3.8 to 86.1 ± 4.1 vs. 94.9 ± 5.9 to 95.3 ± 5.5 kg, NS) or in fat-free mass (from 68.3 ± 2.4 to 67.6 vs. 69.7 ± 2.7 to 69.8 ± 2.9%, NS) in the troglitzone and placebo groups, respectively. Furthermore, none of the subjects receiving troglitazone reported any side effects or had significant abnormalities in safety biochemistry.

Fasting and AUC plasma glucose levels during OGTT measurements were unchanged after troglitzone. In contrast, both fasting and AUC plasma insulin decreased significantly during treatment with troglitazone, as did fasting plasma triglyceride (Table 1). When expressing insulin secretion in relation to insulin sensitivity by means of the disposition, index insulin secretion was not altered by troglitzone (Table 1).

**Clamp measurements**

Plasma glucose concentrations were similar in the troglitzone and placebo groups during both the basal (from 5.6 ± 0.2 to 5.6 ± 0.2 vs. 5.8 ± 0.1 to 5.8 ± 0.1 mmol/l, NS) and insulin-stimulated (from 5.3 ± 0.1 to 5.3 ± 0.1 vs. 5.4 ± 0.1 to 5.3 ± 0.1 mmol/l, NS) steady-state periods. Plasma insulin concentrations during the last 30 min of the clamp study were slightly, but not significantly, lower in the troglitzone group than in the placebo group, especially at the second clamp, reflecting the reduced basal insulin levels after troglitzone (from 389.9 ± 20.8 to 370.4 ± 13.2 vs. 439.3 ± 25.6 to 438.4 ± 23.7 pmol/l, NS). In addition, troglitzone increased insulin-stimulated free fatty acid suppression (from 0.06 ± 0.01 to 0.02 ± 0.01 mmol/l, P < 0.02). However, when compared with placebo (from 0.06 ± 0.02 to 0.06 ± 0.02 mmol/l), it did not achieve statistical significance.

Troglitzone significantly increased $R_d$ during insulin stimulation, whereas
endogenous glucose production, basal and insulin stimulated, remained unchanged. Basal rates of glucose oxidation and glycolytic flux were similar in the two groups after treatment, whereas during insulin stimulation both indirect calorimetry and glycolytic flux measurements showed a trend toward increased glucose oxidation after troglitazone but not to a level of statistical significance. Quantitatively, the improved glucose metabolism during troglitazone treatment was mainly found in the glucose storage pathway of glucose metabolism (Table 2).

**Glycogen synthase activities**

Troglitazone treatment did not result in a concomitant increase in insulin-

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**Table 1—Comparison of fasting metabolic parameters and oral glucose tolerance assessments before and after treatment with placebo or troglitazone**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Before</th>
<th>Placebo After</th>
<th>Troglitazone Before</th>
<th>Troglitazone After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.9 ± 0.1</td>
<td>5.9 ± 0.1</td>
<td>5.9 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>73.6 ± 11.0</td>
<td>73.3 ± 6.5</td>
<td>70.3 ± 6.9</td>
<td>52.2 ± 5.8*</td>
</tr>
<tr>
<td>Fasting plasma C-peptide (pmol/l)</td>
<td>783.1 ± 77.3</td>
<td>742.8 ± 56.2</td>
<td>830.3 ± 57.4</td>
<td>735.8 ± 66.3*</td>
</tr>
<tr>
<td>Fasting plasma FFA (mmol/l)</td>
<td>0.41 ± 0.06</td>
<td>0.45 ± 0.06</td>
<td>0.49 ± 0.04</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>Fasting plasma triglyceride (mmol/l)</td>
<td>2.22 ± 0.44</td>
<td>2.35 ± 0.46</td>
<td>1.86 ± 0.33</td>
<td>1.38 ± 0.27‡†</td>
</tr>
<tr>
<td>OGTT AUC glucose 0–180 min (mmol/l)</td>
<td>7.8 ± 0.3</td>
<td>8.0 ± 0.5</td>
<td>7.8 ± 0.5</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>OGTT AUC insulin 0–180 min (pmol/l)</td>
<td>313.8 (218.2–451.2)</td>
<td>353.9 (208.3–601.3)</td>
<td>335.7 (230.9–488.1)</td>
<td>277.4 (179.4–428.8)</td>
</tr>
<tr>
<td>Disposition index (SecretionOGTT × M × 10³)</td>
<td>1.66 (1.20–2.31)</td>
<td>1.79 (1.38–2.33)</td>
<td>1.61 (1.00–2.60)</td>
<td>1.84 (1.17–2.91)</td>
</tr>
</tbody>
</table>

Data are means ± SE or means (95% CI). *P < 0.02 vs. pretreatment; †P < 0.005 vs. pretreatment; ‡P < 0.01 vs. placebo; §P < 0.05 vs. pretreatment; ||P < 0.05 vs. placebo.

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**Table 2—Rates of glucose and lipid metabolic parameters during clamp studies**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo Before</th>
<th>Placebo After</th>
<th>Troglitazone Before</th>
<th>Troglitazone After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>149.5 ± 23.3</td>
<td>154.6 ± 23.5</td>
<td>164.5 ± 24.3</td>
<td>218.2 ± 32.1*†</td>
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<tr>
<td>Endogenous glucose production</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>96.7 ± 4.2</td>
<td>91.5 ± 3.3</td>
<td>92.6 ± 5.4</td>
<td>95.5 ± 2.8</td>
</tr>
<tr>
<td>Insulin</td>
<td>45.4 ± 4.5</td>
<td>42.5 ± 4.8</td>
<td>38.6 ± 5.0</td>
<td>46.8 ± 4.8</td>
</tr>
<tr>
<td>Total glucose disposal (Rd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>99.7 ± 3.9</td>
<td>93.1 ± 3.2</td>
<td>93.8 ± 4.9</td>
<td>95.4 ± 3.1</td>
</tr>
<tr>
<td>Insulin</td>
<td>197.1 ± 20.0</td>
<td>200.8 ± 20.8</td>
<td>208.3 ± 23.7</td>
<td>263.5 ± 30.4*‡</td>
</tr>
<tr>
<td>Glucose oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>73.9 ± 10.3</td>
<td>65.6 ± 6.3</td>
<td>73.5 ± 10.2</td>
<td>63.3 ± 7.7</td>
</tr>
<tr>
<td>Insulin</td>
<td>112.8 ± 5.7</td>
<td>107.8 ± 8.2</td>
<td>125.2 ± 11.6</td>
<td>136.8 ± 12.4</td>
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<tr>
<td>Lipid oxidation</td>
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</tr>
<tr>
<td>Basal</td>
<td>39.8 ± 4.5</td>
<td>48.8 ± 2.9§</td>
<td>36.9 ± 5.3</td>
<td>40.6 ± 5.1</td>
</tr>
<tr>
<td>Insulin</td>
<td>25.0 ± 2.8</td>
<td>27.7 ± 3.6</td>
<td>16.7 ± 5.4</td>
<td>13.0 ± 5.9</td>
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<tr>
<td>Total energy expenditure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.75 ± 0.01</td>
<td>0.78 ± 0.01</td>
<td>0.71 ± 0.03</td>
<td>0.70 ± 0.02</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.73 ± 0.01</td>
<td>0.72 ± 0.01</td>
<td>0.69 ± 0.02</td>
<td>0.70 ± 0.02</td>
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<tr>
<td>Nonoxidative glucose metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>25.9 ± 9.1</td>
<td>27.5 ± 7.0</td>
<td>20.3 ± 10.3</td>
<td>32.1 ± 7.7</td>
</tr>
<tr>
<td>Insulin</td>
<td>84.3 ± 19.6</td>
<td>93.0 ± 15.5</td>
<td>83.1 ± 14.6</td>
<td>126.7 ± 23.4§</td>
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<tr>
<td>Exogenous glycolytic flux</td>
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<td></td>
</tr>
<tr>
<td>Basal</td>
<td>72.2 ± 8.1</td>
<td>70.6 ± 6.2</td>
<td>72.5 ± 5.1</td>
<td>73.7 ± 5.5</td>
</tr>
<tr>
<td>Insulin</td>
<td>110.0 ± 7.7</td>
<td>112.9 ± 8.4</td>
<td>108.4 ± 7.0</td>
<td>117.5 ± 6.1</td>
</tr>
<tr>
<td>Glucose storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>87.1 ± 16.7</td>
<td>87.9 ± 15.7</td>
<td>99.9 ± 17.9</td>
<td>146.0 ± 25.3†‡</td>
</tr>
</tbody>
</table>

Data are means ± SE. Steady-state rates of metabolic parameters are expressed as mg · m⁻² ·min⁻¹, except for total energy expenditure, which is expressed as kcal · m⁻² ·min⁻¹. *P < 0.005 vs. pretreatment; †P < 0.01 vs. placebo; ‡P < 0.02 vs. placebo; §P < 0.05 vs. pretreatment; ||P < 0.02 vs. pretreatment.
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stimulated fractional (0.1/10 mmol/l G6P) glycogen synthase activity ($FV_{0.1}$ G6P: from 0.43 ± 0.03 to 0.43 ± 0.04 vs. 0.42 ± 0.04 to 0.47 ± 0.03%, NS). In addition, the insulin-stimulated increments in fractional velocities over basal values were similar in the two groups, as were glycogen synthase activities at saturating G6P concentrations (10 mmol/l G6P).

CONCLUSIONS — First-degree relatives of patients with type 2 diabetes have an ∼40–50% risk of developing overt diabetes and as such represent a relevant population to test whether interventional procedures can modify the metabolic abnormalities believed to precede the disease. In the present study, we have targeted insulin resistance pharmacologically by treating glucose-tolerant first-degree relatives with the insulin action enhancer troglitazone. Our results clearly demonstrate that insulin resistance can be substantially ameliorated with no changes observed in their normal glucose tolerance. The improved insulin action was associated with a reduction in the circulating triglyceride level and a slightly increased insulin-mediated suppression of free fatty acids, emphasizing the important regulatory role of troglitazone on lipid availability.

Previous studies in groups of subjects with IGT (21,22,39), or in which the majority have IGT (20), have shown reduced fasting insulin concentrations and a lower insulin response to a glucose load with 400 mg troglitazone daily. In the present study, fasting insulin concentrations fell by ∼26% and the OGTT insulin area by ∼18%. On average, these reductions in insulin variables are of the same magnitude as previously observed in studies on IGT subjects but, importantly, obtained without a simultaneous improvement in glycemia and furthermore by using a 200-mg dose of troglitazone, which is known to be in the lower end of the dose response curve (40). The reduced β-cell demand could play a role in delaying or preventing type 2 diabetes in first-degree relatives, and given their known high risk for diabetes, it may even be beneficial to attack insulin resistance in this group at an earlier stage, i.e., before glucose tolerance starts to deteriorate. This could also have implications for the development of premature arteriosclerosis, which is known to be already present in subjects with IGT (1). The fact that glucose curves before and after were almost completely superimposed despite a fall in insulin area during troglitazone treatment suggests that troglitazone has little or no effect on insulin secretion in glucose-tolerant subjects. In line with this, we were not able to detect any effect of troglitazone on insulin secretion using the disposition index.

Contrary to observations in patients with type 2 diabetes (19), the relatives did not gain weight during treatment with troglitazone. Most likely this is due to the relatively short duration of the study and the fact that these subjects did not experience a reduction in hyperglycemia. On the other hand, in subjects with normal glucose tolerance, an increased insulin sensitivity will lead to an almost proportional reduction in insulin output, which would tend to counterbalance a possible weight gain.

It is known that ∼90% of type 2 diabetic subjects are obese at diagnosis and that a majority of these subjects have been overweight several years before deterioration of glucose tolerance. Furthermore, in the longitudinal study by Martin et al. (3), relatives who developed diabetes were significantly heavier than those who did not. In the present study, we therefore deliberately included overweight first-degree relatives, which in our view confers a higher risk for later type 2 diabetes than lean offspring at the same age. Our aim was not to show that the beneficial effects of troglitazone in first-degree relatives are caused by an effect on putative diabetes, insulin resistance, or obesity genes or that treatment should significantly heavier than those who did not. In the present study, we therefore deliberately included overweight first-degree relatives, which in our view confers a higher risk for later type 2 diabetes. The rational of including overweight relatives in preventive studies seems to be consistent with a recent study showing that obesity doubled the risk of development of diabetes in first-degree relatives (41). Moreover, in the same study it was shown that obesity had a much smaller impact on the development of diabetes in subjects with no family history of diabetes. To our knowledge, only one study has evaluated the effects of troglitazone in obese subjects with normal glucose tolerance and no family history of diabetes (42). This study was uncontrolled without a placebo group, and three times the dose of troglitazone was used. Also subjects were much more obese. During treatment with troglitazone these investigators found positive effects on fasting insulin levels and glucose disposal in the same magnitude or slightly lower than in the present study.

Treatment with troglitazone increased insulin-stimulated glucose disposal ∼47%, and by using the tritiated water technique, we could demonstrate that this improvement was mainly found in the glucose storage pathway of glucose metabolism, i.e., glycogen synthesis. Furthermore, insulin action on endogenous glucose production, both basal and during clamping, was similar in response to troglitazone and placebo treatment, which is consistent with a previous study of normoglycemic subjects (43). Glycogen formation is catalyzed by the enzyme glycogen synthase, which can be activated either covalently by insulin through mitogen-activated protein kinase or allosterically by G6P (43). In the present study, the increased glucose storage was not accompanied by a simultaneous increase in the insulin-mediated activation of glycogen synthase, suggesting a role for G6P in increasing the flux through the glycogen synthetic pathway. This notion is supported by a study (44) in which troglitazone increased glucose transport activity and intramuscular G6P concentrations in type 2 diabetic subjects during hyperglycemic conditions through the nuclear magnetic resonance technique. In line with this, the improved insulin-mediated free fatty acid suppression after troglitazone observed in our study could be an important mechanism by which troglitazone improves glucose metabolism. Thus, increased free fatty acid concentrations have been shown to inhibit skeletal muscle glucose transport and phosphorylation (45,46) and to reduce glycogen synthesis (47). It follows that the troglitazone-mediated reductions in circulating lipids could alleviate these unfavorable effects. This notion could also be consistent with our recent finding (25,48) of increased protein kinase B phosphorylation after troglitazone and the possible role for this kinase among others on glucose transport.

Our finding that troglitazone does not affect insulin-stimulated glycogen synthase activity is partly in variance with a study (49) in human muscle cell culture in which the acute and chronic effect of
troglitazone on glucose uptake and glycogen synthase activity was investigated. In both situations, glucose uptake increased whereas glycogen synthase activity responded only after chronic treatment with dose-dependent increases in both insulin-independent and -stimulated FVs. The reason for this discrepancy is not quite clear, but could relate to the supraphysiological conditions in the cell cultures where troglitazone concentrations reached a level 10–50 times higher than in our in vivo study (40) and insulin concentrations reached a level ~100 times higher. Furthermore, we note that in another study (52) using human skeletal muscle cell culture, chronic troglitazone treatment was associated with alterations in cell morphology (transdifferentiation) and an increase in adipocyte markers, such as adipocyte lipid binding protein.

The beneficial effects of troglitazone on circulating lipids in the present study are consistent with previous findings in both subjects with IGT (21,39) and type 2 diabetes (18,44). They support the concept that troglitazone (PPARγ agonists) improves insulin responsiveness via binding to PPARγ receptors in the adipocyte where these receptors are expressed most prominently (6) and promotes storage of free fatty acids in adipocyte triglycerides (7,8). However, the improvement in glucose metabolism could also include altered expression of adipocyte derived mediators of insulin resistance (12–14) and, as recently proposed, repartitioning of lipids away from muscle into adipose tissue (5,11). The latter concept would be of special importance due to nuclear magnetic resonance findings showing increased intramyocellular triglyceride in insulin-resistant offspring of type 2 diabetic patients (51), although it remains to be demonstrated whether this is a primary or secondary phenomenon. Interestingly, the sequestration of lipids into adipocytes may also prevent B-cell deterioration by reducing islet fat, as shown morphometrically in islets from prediabetic rodents (52).

In summary, we provide evidence that treatment with troglitazone (PPARγ agonists) in young, overweight, but normal glucose tolerant first-degree relatives of patients with type 2 diabetes to a great degree reverses insulin resistance with the prospect of postponing or preventing the development of overt type 2 diabetes in this high-risk population. Moreover, we speculate that this therapeutic potential may work even better if used before glucose tolerance starts to deteriorate. To answer these questions, however, long-term studies are needed.

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References

Effects of troglitazone in relatives


