Rosiglitazone Improves Insulin Resistance, Lipid Profile and Promotes Adiposity in a Genetic Model of Metabolic Syndrome X

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Abstract. RSG is a member of the TZD group of drugs widely used in treatment of type 2 diabetes. The underlying mechanism of TZD action in insulin-sensitive tissues is not fully understood. In this study we show that 14-day RSG administration in a new rodent model of metabolic syndrome X, polydactylous rat strain (PD/Cub), substantially improves its lipid profile (serum TGs 4.20 ± 0.23 vs 2.34 ± 0.14 mmol/l, P < 0.0001; FFA 0.46 ± 0.05 vs 0.33 ± 0.02 mmol/l, P = 0.017), diminishes the liver TG depots (15.76 ± 0.60 vs 8.44 ± 0.55 µmol/g, P < 0.0001), serum insulin concentrations (1.10 ± 0.08 vs 0.63 ± 0.02 nmol/l, P < 0.0001) and promotes visceral adiposity (adiposity index 1.28 ± 0.03 vs 1.85 ± 0.07, P < 0.0001). No changes were observed in serum or liver concentrations of cholesterol. Concomitantly, both basal and insulin-stimulated glycogen synthesis in red-fibre type muscle (m. soleus) was enhanced, as well as glucose uptake into adipose tissue. However, glucose oxidation in soleus (basal and insulin-stimulated) remained unchanged. In consent with previously published data we suggest the current pharmacogenetic study as a further proof of substantial influence of genetic background on the physiological outcome of TZD therapy.

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the effect of RSG administration on the metabolic profile and tissue glucose disposal in a new inbred model of metabolic syndrome X, polydactylous rat strain (PD/Cub).

**Material and Methods**

**Animals**

The polydactylous rat strain (PD/Cub) is a highly inbred rat strain (F > 70, verified by several total genome scans) kept since 1969 at the Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague. It carries a mutant allele of the Lx gene, which gives rise to the polydactyly-luxate syndrome (Křen, 1975). It has been exploited as a model of limb development and teratology (Křen et al., 1996), hypertriglyceridemia (Vrana et al., 1993), and it was established as a model for metabolic syndrome X (Šedová et al., 2000).

Male rats (3 months of age) of the PD/Cub strain were randomly divided into two groups (N = 7 and N = 8). The rats had free access to water and were fed standard chow followed with either 14 days of high-sucrose diet (70% calories as sucrose) or the sucrose standard chow followed with either 14 days of high-sucrose diet (70% calories as sucrose) or the sucrose diet combined with RSG (Avandia, 0.4 mg/100 g total body weight).

**Metabolic measurements**

The oral glucose tolerance test (OGTT) was performed after overnight fasting. Blood for glycemia determination was drawn from the tail at intervals of 0, 30, 60 and 120 min after intragastric glucose administration to conscious rats (3 g/kg total body weight, 30% aqueous solution). Commercially available analytical kits were employed to determine plasma glucose and serum triglyceride (TG) concentrations (Lachema, Brno, Czech Republic). Serum free fatty acids (FFA) were determined using an acyl-CoA oxidase-based colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany). At the end of the experiment, the rats were sacrificed and the weights of liver, kidneys, epididymal fat pads (EFP) and retroperitoneal fat pads (RFP) were determined.

**Insulin-stimulated glucose oxidation and glycogen synthesis.** Basal and insulin-stimulated glucose incorporation into glycogen and CO₂ was determined in isolated soleus muscle as described previously (Vrána and Kazdová, 1970). After decapitation, the soleus muscles were attached to a stainless steel frame in situ at 37°C, separated from other muscles and tendons and immediately incubated for 2 h in Krebs-Ringer bicarbonate buffer, at 37°C, gas phase 95% O₂ + 5% CO₂, pH 7.4, that contained 5.5 mM unlabelled glucose, 0.1 μCi/ml of 14C-U glucose, and 3 mg/ml BSA (Armour, Fraction V) with or without 250 μU/ml insulin. After 2-h incubation, 0.3 ml of 1 M hyamine hydroxide were injected into the central compartment of the incubation vessel and 0.5 ml of 1 M H₂SO₄ into the main compartment to liberate CO₂. The vessels were incubated for another 30 min, the hyamine hydroxide was then quantitatively transferred into a scintillation vial containing 10 ml of toluene-based scintillation fluid for counting of radioactivity. For measurement of glucose incorporation into glycogen, glycogen from the soleus muscles was extracted as described previously (Vrána and Kazdová, 1970).

**Liver TG and cholesterol measurements.** For determination of TGs and cholesterol in liver, tissues were powdered under liquid N₂ and extracted for 16 h in chloroform : methanol, after which 2% KH₂PO₄ was added and the solution centrifuged. The organic phase was removed and evaporated under N₂. The resulting pellet was dissolved in isopropyl alcohol, and TG and cholesterol content was determined by the enzymatic assay (Lachema, Brno, Czech Republic).

**Statistical analysis**

The data for all traits were first subjected to the Brown-Forsythe test of homogeneity of variances. Where proved significant, non-parametric testing was performed. Otherwise, the experimental groups were compared using Student’s t-test. Data are expressed as mean ± S.E.M.

**Results**

**Effect of RSG on the metabolic profile of PD/Cub**

The group treated with RSG displayed significantly lower serum concentrations of TGs (both fasting and non-fasting), FFAs and insulin (Table 1). We did not observe any differences in cholesterol and fasting glucose levels. The area under curve (AUC) of the OGTT test (Fig. 1) was lower in RSG-treated rats, though the difference did not reach statistical significance. However, the insulin/glucose ratio was significantly lower in the RSG-treated group (0.205 ± 0.017 vs 0.117 ± 0.006, P < 0.001). During the course of OGTT, glucose concentrations in the RSG-treated group were lower only in 60th and 120th min after the glucose load (P = 0.04 and P = 0.005, respectively).
Effect of RSG on fat depots

Although the total body weight did not change in either of the experimental groups, the group treated with RSG had a higher adiposity index (calculated as EFP weight / 100 g total body weight) and a lower relative liver weight, and we found no difference in the relative weight of RFP. RSG administration resulted in an almost two-fold decrease of liver TG content with no effect on liver cholesterol (Table 2).

Effect of RSG on insulin action in tissues

The net rate of glucose incorporation into glycogen (referred to as Glycogenesis) and the rates of CO₂ production from glucose (referred to as Glucose oxidation) in soleus, the mostly oxidative muscle, and the insulin-stimulated incorporation of ¹⁴C-U glucose into the lipids of adipose tissue (Lipogenesis) were determined in order to directly assess the influence of RSG on insulin sensitivity and glucose disposal in these tissues.

RSG administration resulted in a significant increase of basal and insulin-stimulated lipogenesis as well as in augmentation of the insulin effect on lipogenesis (measured as % increase compared to baseline; 102.4% ± 11.0% vs 152.9% ± 18.8%, P = 0.04). The glycogenesis (basal and insulin-stimulated) was also substantially increased, though the insulin response (%) was similar. On the other hand, any of the parameters of glucose oxidation did not differ between the two treatment groups (Table 3).

Discussion

In this study we show that 14-day RSG administration in a new rodent model of metabolic syndrome X substantially improves its lipid profile, diminishes the liver TG depots, serum insulin concentrations and promotes visceral adiposity. Concomitantly, the glycogenesis and lipogenesis are greatly enhanced, though the extent of and insulin effect on glucose oxidation in red-fibre type muscle (m. soleus) remains unchanged.

In recent years, several studies addressed the question of TZD effect on the glucose disposal in skeletal muscle, yielding ambiguous results. Furnsinn et al. (1999) showed that glycogenesis is stimulated in solei of ZDF obese rats (contrary to the lean controls) fed either BM 13.1258 or BM 15.2054 for 10 days. Glucose oxidation was enhanced in the muscle only by the former of the two TZD. On the contrary, results from the same group with in vitro exposure of m. soleus to up to six types of TZD shows clear inhibition of glucose oxidation (Furnsinn et al., 2000, Brunmair et al., 2001). Finally, Furnsinn and Waldhausl (2002) summarize that the divergent effects on intracellular glucose routing depend on concentration of TZDs and exposure period. Enhanced glycogenesis and insulin effect together with enhanced glucose oxidation in red fibre-type muscle (m. soleus) remains unchanged.

Table 1. Metabolic profile of PD/Cub with P-values for Student’s t-test. Values are shown as mean ± S.E.M. Sucrose...high-sucrose diet; RSG...high-sucrose diet + RSG; f...fasting; nf...non-fasting; ns...not significant.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Unit</th>
<th>Sucrose</th>
<th>RSG</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGs (f)</td>
<td>mmol/l</td>
<td>3.78 ± 0.33</td>
<td>1.90 ± 0.09</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>TGs (nf)</td>
<td>mmol/l</td>
<td>4.20 ± 0.23</td>
<td>2.34 ± 0.14</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mmol/l</td>
<td>1.42 ± 0.08</td>
<td>1.44 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>FFA</td>
<td>mmol/l</td>
<td>0.46 ± 0.05</td>
<td>0.33 ± 0.02</td>
<td>0.017</td>
</tr>
<tr>
<td>Insulin</td>
<td>nmol/l</td>
<td>1.10 ± 0.08</td>
<td>0.63 ± 0.02</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Glucose</td>
<td>mmol/l</td>
<td>5.41 ± 0.16</td>
<td>5.42 ± 0.26</td>
<td>ns</td>
</tr>
<tr>
<td>AUC (OGTT)</td>
<td></td>
<td>856.6 ± 17.82</td>
<td>803.97 ± 19.64</td>
<td>0.072</td>
</tr>
</tbody>
</table>

Fig. 1. Oral glucose tolerance test in PD/Cub fed high-sucrose diet with or without RSG. Black and white circles represent measurements in groups fed high-sucrose diet without and with RSG, respectively. Statistical significance levels are indicated as follows: *P < 0.05.
attributed to normalization of both glycogen synthesis and glycolysis (with no effect of RSG on the insulin level and relatively smaller effect on the whole body glucose disappearance). Unfortunately, none of the above mentioned in vivo studies involved measurement of FFA concentrations, one of the proposed key metabolites in TZD action (Oakes et al., 2001). Jucker et al. (2002) suggest that different TZDs exhibit different responses with regard to glycogen synthesis and glucose oxidation and therefore may control glucose metabolism via different mechanisms. Our results from this and previous studies allow us to add a notion that not only the type of TZD, but also the genetic background they are acting upon determines the physiological outcome of TZD therapy. This is documented by contrasting effects of RSG and pioglitazone (Qi et al., 2002) on glycogen synthesis, glucose oxidation, insulin levels and other metabolic parameters in several inbred strains, i.e. PD/Cub, BN/Cub, BN.SHR4 or SHR, SHR.BN4, SHR.TG19, respectively. In PD/Cub the levels of serum FFA decreased by almost 30% (combined with 46% decrease of liver TG stores and 44% decrease of plasma TG). Most of the generated FFA were presumably taken up by the adipose tissue, substantially increasing visceral adiposity, possibly by inducing TG storage and adipocyte differentiation (Albrektсен et al., 2002). RSG has been so far reported to decrease (Watkins et al., 2002), increase (Boyle et al., 2002) or not affect (Haffner et al., 2002) cholesterol levels. Here, both serum cholesterol level and liver cholesterol content were not changed by RSG, which is in contrast with the effect of PPAR-alpha agonist, fenofibrate observed in the PD/Cub strain (Šeda et al., 2001).

Overall, we have shown that RSG administration in a genetic model of syndrome X, PD/Cub rat, apart from insulinopenic and lipopenic action, exerts a distinct effect on glucose oxidation and glycogen synthesis in oxidative muscle. With the prevalence of metabolic syndrome reaching about 25% in westernized countries and wide-spread use of TZD drugs, deeper understanding of pharmacogenetic interactions determining the outcomes of TZD therapy is becoming a matter of major importance and defined genetic models represent a valuable tool for such studies.

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References


