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

(rosiglitazone / metabolic syndrome X / genetic models / PD/Cub)

O. ŠEDA[1,2], L. KAZDOVÁ[2], D. KŘENOVÁ[1], V. KŘEN[1,3]

[1]Institute of Biology and Medical Genetics, First Faculty of Medicine , Charles University, Prague, Czech Republic

[2]Institute for Clinical and Experimental Medicine, Prague, Czech Republic

[3]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

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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Corresponding author: Vladimír Křen, Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Albertov 4, 128 00 Prague 2, Czech Republic. Tel.: +420 224 968 147; email: vkren@lf1.cuni.cz.

Abbreviations: AUC – area under curve, *Cd36/Fat* – fatty acid translocase, FFA – free fatty acids, OGTT – oral glucose tolerance test, PPARγ – peroxisome proliferator-activated receptor gamma, RSG – rosiglitazone, TG – triglyceride, TZD – thiazolidinedione, ZDF – Zucker diabetic „fatty“ rat.

The thiazolidinedione (TZD) class of drugs are potent peroxisome proliferator-activated receptor gamma (PPARγ) agonists used in therapy of type 2 diabetes and other insulin resistance states (Olefsky et al., 2000). Their efficacy in improving glycemic control is attributed to enhancement of insulin-stimulated glucose disposal in liver, adipose and muscle tissues. However, the underlying mechanism for TZD action remains unclear, the main issues raised by current findings (Cullen and Lorkowski, 2002) include the mode of TZD effect on muscle (direct vs. adipokine-mediated) and the dissociation of PPARγ downstream events (dependence on the type of agonist and elicited biological response).

Recently, we have found that rosiglitazone (RSG) action on carbohydrate and lipid metabolism is substantially blunted in the BN.SHR4 congenic strain, which carries a defective allele of the fatty acid translocase (*Cd36/Fat*) gene (Šeda et al., 2002). By showing distinct effects of pioglitazone on metabolic profiles of SHR, SHR.BN4 congenic and SHR.TG19 transgenic rat strains, Qi et al. (2002) provided further evidence for the necessity of intact *Cd36/Fat* in TZD action. Controversial results concerning hepatic lipid handling are reported from TZD administration in several rodent models of type 2 diabetes and/or obesity, such as C57BL/6J-Lep^{ob}/Lep^{ob} „obese“ mice, ZDF-Lepr^{fa}/Lepr^{fa} „fatty“ rats or KKA-A^{y} and (NZOxNOD)F1 mice (Watkins et al., 2002). In humans, Vestergaard et al. (2001) demonstrated lack of therapeutic effect of RSG in individuals with a genetic defect of the insulin receptor. Altogether, there is a considerable amount of evidence for the presence of a strong pharmacogenetic component in TZD action. Genetically defined models can provide an invaluable resource for determination of alleles/allelic combinations that may precipitate the side-effects of TZD therapy, namely increased adiposity, oedema and rare but serious hepatotoxicity (reviewed by Isley and Oki, 2001, and Lebovitz, 2002). In this study, we analysed

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 (Vrána 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 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 (EFP) and retroperitoneal fat pads (RFP) were determined.

Insulin-stimulated glucose oxidation and glycogen synthesis. Basal and insulin-stimulated glucose incorporation into glycogen and CO_2 was determined in isolated soleus muscle as decribed previously (Vrána et al., 1978). After decapitation, the soleus muscles were attached to a stainless steel frame *in situ* at *in vivo* length, separated from other muscles and tendons and immediately incubated for 2 h in Krebs-Ringer bicarbonate buffer, at 37°C, gas phase 95% O_2 + 5% CO_2, pH 7.4, that contained 5.5 mM unlabelled glucose, 0.1 μCi/ml of ^{14}C-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 H_2SO_4 into the main compartment to liberate CO_2. 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).

Insulin-stimulated lipogenesis. Basal and insulin-stimulated incorporation of ^{14}C-U glucose into total lipids of rat adipose tissue *in vitro* (lipogenesis) was determined. In short, after decapitation, distal parts of the epididymal adipose tissue (200 mg) were incubated in Krebs-Ringer bicarbonate buffer under conditions described above. Total adipose tissue lipids were extracted according to Folch et al. (1957) and the radioactivity was determined 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 N_2 and extracted for 16 h in chloroform : methanol, after which 2% KH_2PO_4 was added and the solution centrifuged. The organic phase was removed and evaporated under N_2. 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).

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.

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

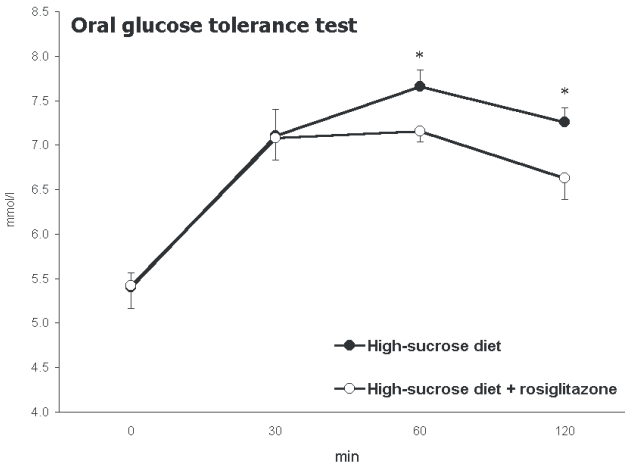

Fig. 1. Oral glucose tolerance test in PD/Cub fed high-sucrose diet with or without RSG. Black and white circles represent measurements in groups fed high-sucrose diet without and with RSG, respectively. Statistical significance levels are indicated as follows: *P < 0.05.

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 CO_2 production from glucose (referred to as **Glucose oxidation**) in soleus, the mostly oxidative muscle, and the insulin-stimulated incorporation of ^{14}C-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 Waldahausl (2002) summarize that the divergent effects on intracellular glucose routing *in vitro* depend on concentration of TZDs and exposure period. Enhanced glycogenesis and insulin effect together with enhanced glucose oxidation in red fibre-type musle was observed in ZDF rats fed troglitazone for 6 weeks in a study by Sreenan et al. (1999). Most recently, using the *in vivo* ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectroscopic approach, Jucker et al. (2002) found that in RSG-treated (7 days of administration) ZDF rats, increased insulin-stimulated glucose disposal in skeletal muscle can be

Table 2. Adiposity and adipose tissue depots in 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; ns...not significant.

Phenotype	Unit	Sucrose	Rosiglitazone	P
Total body weight	g	333.1 ± 5.2	346.5 ± 8.2	*ns*
Adiposity index		1.28 ± 0.03	1.85 ± 0.07	**< 0.0001**
Retroperitoneal fat weight/100 g		0.98 ± 0.04	1.07 ± 0.07	*ns*
Liver weight/100 g		3.41 ± 0.08	2.99 ± 0.08	**0.004**
Liver cholesterol content	mmol/g	8.32 ± 0.25	8.25 ± 0.53	*ns*
Liver TG content	mmol/g	15.76 ± 0.60	8.44 ± 0.55	**< 0.0001**

*Table 3. Insulin-mediated glucose utilization in adipose and muscle tissues in 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; ns...not significant; *...nmol glucose/mg protein/2 hours; #...nmol glucose/g/2 h. Methods abbreviated as Lipogenesis, Glycogenesis and Glucose oxidation are described in the text.*

Phenotype	Unit	Sucrose	RSG	P
Lipogenesis (insulin -)	*	31.56 ± 3.89	43.05 ± 2.72	**0.028**
Lipogenesis (insulin +)	*	62.14 ± 5.69	107.96 ± 9.32	**0.001**
Glycogenesis (insulin -)	#	599.59 ± 49.80	1178.59 ± 107.55	**0.0005**
Glycogenesis (insulin +)	#	1096.00 ± 168.22	1704.81 ± 121.76	**0.011**
Glucose oxidation (insulin -)	#	151.47 ± 5.22	162.50 ± 16.92	*ns*
Glucose oxidation (insulin +)	#	207.74 ± 13.59	211.59 ± 16.53	*ns*

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 (Albrektsen 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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