Troglitazone (CS-045) Normalizes Hypertriglyceridemia and Restores the Altered Patterns of Glucose-Stimulated Insulin Secretion in Dyslipidemic Rats

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Rats fed a sucrose-rich diet ([SRD] 63% wt/wt) up to 270 days develop stable hypertriglyceridemia, impaired glucose tolerance, and insulin insensitivity. The aim of the present study is to investigate whether the hypoglycemic agent troglitazone introduced as a pharmacologic intervention could improve and/or reverse the whole-body insulin insensitivity and related abnormalities present after feeding normal rats with a SRD long-term. For this purpose, male Wistar rats were fed a SRD for 210 days. While half of the animals continued with this diet for up to 270 days, troglitazone (0.2 g/dL wt/wt) was added to the SRD of the other half for up to 270 days. Troglitazone markedly reduced in vivo the hepatic triglyceride secretion rate (TGSR) and enhanced its removal from the circulation, leading to a normalization of plasma triglyceride levels. It also normalized the whole-body peripheral insulin resistance, the glucose homeostasis, and the elevated free fatty acids (FFAs) without detectable changes in plasma insulin levels. The clear alteration of the biphasic pattern of glucose-stimulated insulin secretion in the in vitro perfused β-cell islets of rats fed the SRD long-term (270 days) was also completely normalized when the SRD was supplemented with troglitazone for 2 months. The normalization of the altered patterns of glucose-stimulated insulin secretion, as well as the enhancement of peripheral insulin sensitivity without detectable changes in plasma insulin, might be largely a result of the significant action of troglitazone in the decrease of circulating lipids and enhancement of whole-body glucose metabolism.

ENVIRONMENTAL NONGENETIC FACTORS such as a diet high in refined sugar can produce a state of impaired glucose homeostasis, insulin resistance, and hypertriglyceridemia.[1,2] Over the past years, our laboratory has reported that in normal rats fed a sucrose-rich diet (SRD), a 3-step metabolic syndrome is clearly identified as follows according to the amount and type of carbohydrate source, the length of time during which the SRD is administered, and the age of the animal: (1) induction period (3 to 5 weeks on SRD) characterized by hypertriglyceridemia, a moderate increase of plasma free fatty acid (FFA) levels, hyperinsulinemia, and impaired glucose tolerance to intravenous (IV) glucose challenge; (2) adaptation period (5 to 8 weeks on SRD) featuring a spontaneous normalization of the aforementioned parameters by a yet unknown mechanism; and (3) recurrence period (8 to 15 weeks on SRD) involving moderate hyperglycemia with normoinsulinemia, hypertriglyceridemia, sustained increase of plasma FFA, and severe glucose intolerance (insulin insensitivity).[3-6] From then on, we observed a steady state of hypertriglyceridemia, elevated plasma FFAs, impaired glucose homeostasis without detectable changes in circulating insulin levels, and a more pronounced whole-body insulin insensitivity when the feeding period was extended up to 30 weeks.[7]

The pharmacologic agent troglitazone (also known as CS-045), a thiazolidinedione derivate, has been shown to decrease circulating lipid levels (mainly triglyceride and FFA) and enhance insulin-stimulated glucose metabolism without stimulating β-cell insulin secretion.[8] Most of the available studies on the metabolic effects of troglitazone on the experimental model of hypertriglyceridemia, abnormal glucose homeostasis, and hyperinsulinemia induced by a high-fructose or -sucrose intake were performed during the induction period. Those studies showed that the metabolic abnormalities were all completely prevented by concomitant administration of the agent and the fructose diet.[9,10] At present, we are unaware of any report concerning the effect of troglitazone introduced as a pharmacologic intervention after a steady state of dyslipidemia and whole-body insulin insensitivity is present in normal rats fed with a SRD for a long time (recurrence period, SRD from 15 to 30 weeks on). This is particularly interesting because, as already mentioned, (1) a different hormonal and metabolic milieu evolves from the early (3 to 5 weeks) to late (15 to 30 weeks) stages of hypertriglyceridemia[3] and (2) several metabolic trails that developed only after feeding rats long-term on a SRD, such as hypertriglyceridemia, increased plasma FFA, moderate hyperglycemia, and insulin insensitivity, are also present in patients with type 2 diabetes or lipid disorders associated with glucose intolerance. This opened new avenues of research aiming at the control and treatment of these metabolic disorders.

Therefore, this study was undertaken to assess whether the hypoglycemic agent troglitazone can improve and/or reverse the whole-body insulin insensitivity and related abnormalities present after feeding normal rats with a SRD long-term. We studied (1) ''in vivo'' peripheral insulin sensitivity (euglycemic-hyperinsulinemic clamp), triglyceride secretion, and triglyceride fractional rates of removal from the circulation and (2) ''in vitro'' insulin secretion patterns from perifused isolated islets.

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MATERIALS AND METHODS

Animals and Diets

Male Wistar rats initially weighing 170 to 185 g and purchased from the National Institute of Pharmacology in Buenos Aires, Argentina, were used in the present study. The animals were housed and initially fed a standard rat laboratory chow (Ralston Purina, St Louis, MO) and water in an animal room with controlled temperature (22° ± 1°C), humidity, and air flow and a fixed 12-hour light/dark cycle (light from 7 AM to 7 PM). After a 1-week acclimatization period, the rats were randomly divided into 2 groups, control and experimental. The experimental group was fed a semisynthetic sucrose-rich (63% wt/wt) diet (SRD) and the control rats received the same semisynthetic diet with the only exception that sucrose was replaced by starch (63% wt/wt, control diet [CD]). The experimental group received the SRD for 210 days, after which, the animals were randomly divided into 2 subgroups. One subgroup (SRD + troglitazone) continued receiving the SRD up to day 270, to which the hypoglycemic agent (a generous gift from Sankyo, Tokyo, Japan) was added daily at 0.2 g/dL wt/wt from day 210 to day 270. The second subgroup (SRD) continued with the SRD up to 270 days. The control rats received the CD throughout the experimental 270-day period. In the latter 2 above-mentioned groups, starch 0.2 g/dL wt/wt was added as a placebo from day 210 to day 270. Troglitazone was freshly mixed in small amounts of food every 2 to 3 days and stored at 4°C. Preliminary results showed that the stable hypertriglyceridemia, hyperglycemia, and normoinsulinemia recorded in rats fed the experimental diet for 210 days (30 weeks) were still present at 270 days on the SRD. The values were as follows (mean ± SEM, n = 6, CD *v* SRD): triglyceride, 0.52 ± 0.04 *v* 1.60 ± 0.15 mmol/L; glucose, 6.60 ± 0.09 *v* 8.15 ± 0.13 mmol/L; and insulin, 49.7 ± 3.8 *v* 53.0 ± 4.6 μU/mL. The preparation, nutritional components, and handling of the diets have been previously reported in detail.[11] All diets provided approximately 15.28 kJ/g chow and were available ad libitum. The weight of each animal was recorded twice per week during the experimental period. In a separate experiment, the individual caloric intake and weight gain of at least 8 animals in each group were assessed daily. On the day of the experiments, food was removed at 7 AM unless otherwise indicated and the experiments were performed between 8 AM and noon. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Santa Fe, Argentina.

Analytic Methods

The rats were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg body weight). Blood samples were obtained from the jugular vein and rapidly centrifuged at 4°C. The serum samples were either immediately analyzed or stored at −20°C and examined within the following 3 days. Plasma triglyceride,[12] FFA,[13] and glucose[14] were determined by spectrophotometric methods. The immunoreactive insulin level was measured by the method of Herbert et al.[15] The insulin assays were calibrated against rat insulin standard (Novo, Bagsvaerd, Denmark). The livers were rapidly removed from the animals, and the homogenates of frozen liver powder were used for the determination of triglyceride[12] and for total protein with the method of Lowry et al.[16] The liver marker enzymes aspartate transaminase (AST) and alanine transaminase (ALT) were measured by the standard spectrophotometric methods.[14]

Triglyceride Secretion Rate

The triglyceride secretion rate (TGSR) was evaluated using an alkaryl polyether anionic detergent (Triton WR 1339) that blocks the removal of intravascular lipoprotein with density <1.006 g/mL. Briefly, anesthetized fasting rats (16 to 18 hours) were injected IV with Triton WR1339 (600 mg/kg body weight dissolved in 0.9% NaCl), and the subsequent increase in triglycerides was used to calculate TGSR secretion rates into the plasma. To determine the time course of triglyceride accumulation in plasma, blood samples were drawn before and at 60 and 120 minutes thereafter for triglyceride measurements. The amount of Triton necessary to provide maximal inhibition of triglyceride removal from plasma and the linearity of the rate of secretion were previously determined.[4] The TGSR was calculated from the linear increase of triglyceride versus time. In a separate experiment, the plasma volume was determined by the Evans blue dye dilution technique[17] in rats that were fed 270 days with their respective diet. More details of the methodology are presented elsewhere.[4,18]

IV Fat Tolerance Test

The IV fat tolerance test (IVFTT), which involves an artificial fat emulsion, is a convenient tool for the study of triglyceride dynamics in normal and abnormal metabolic states. Intralipid, a soybean oil 10% IV fat emulsion, was found to be a useful tracer for the study of the fractional turnover rate of circulating triglyceride.[18] The IVFTT was performed in anesthetized rats fasted 16 to 18 hours as previously described by our group.[18] Briefly, the rats were injected IV with 0.1 mL/100 g body weight of 10% Intralipid (lot MR 50818 B; Kabivitrum, Alameda, CA) and serial blood samples (approximately 0.2 mL) were drawn immediately before and over the next 30 minutes thereafter. The hematocrit changed from 0.46 ± 0.008 at 0 minutes to 0.45 ± 0.006 at 30 minutes after the injection (n = 6). The intralipid level in plasma was measured by nephelometry after suitable dilution with sodium chloride 9 g/L. The first-order rate constant (K_2 values, percent per minute) of elimination of the fat emulsion from the bloodstream (fractional removal rate) was calculated by the least-squares method. Details of the methodology are given elsewhere.[18]

Euglycemic Clamp Studies

Insulin sensitivity was measured using the euglycemic-hyperinsulinemic clamp technique as previously described.[11] Briefly, the rats were anesthetized after a 5-hour fasting period. Then, a blood sample was obtained, on which glucose and insulin levels were assessed. Afterward, an infusion of highly purified porcine neutral insulin (Actrapid; Novo Industry, Denmark) at 0.8 $U \cdot kg^{-1} \cdot h^{-1}$ for 2 hours was started. Insulin was infused through one limb of a double-lumen cannula connected to the left jugular vein. Blood samples for glucose assays were taken at 5- to 10-minute intervals from the right jugular vein. The blood glucose concentration was measured using the glucose oxidase method[14] (Glucose Accutrend Sensor; Boehringer Mannheim Biochemicals, Buenos Aires, Argentina) within 2 minutes after the samples were obtained. Glycemia was maintained at an euglycemic level by injecting 0.2 g/mL glucose solution at a variable rate through the second limb of the double-lumen cannula. The glucose infusion began 5 minutes after the insulin infusion started. The glucose infusion rate (GIR) during the second hour of the clamp was taken as the net steady state of whole-body glucose. In all studies, blood samples (0.3 mL) for insulin determination[15] were obtained at 60, 90, and 120 minutes.

Perifusion of Isolated Islet

The rats were fasted for 12 hours and decapitated, and the islets were isolated by collagenase digestion and collected under a stereoscopic microscope.[19] After washing the islets twice with Krebs-Ringer bicarbonate (KRB) buffer, groups of 30 to 40 islets isolated from each rat were loaded in a 13-mm chamber containing a 5-μm nylon membrane filter. Islets were perifused with KRB buffer containing NaCl 118 mmol/L, KCl 4.7 mmol/L, $MgSO_4$ 1.3 mmol/L, $CaCl_2$ 1.2 mmol/L, $NaHCO_3$ 25 mmol/L, glucose 3 mmol/L, bovine serum albumin 0.25 mg/mL, dextran-70 4 mg/dL, pH 7.4 at 37°C (constantly gassed with

Table 1. Body Weight Gain and Caloric Intake in Rats Fed on the Different Diets

Diet	Initial Body Weight (g, day 1)	Body Weight (g, day 210)	Caloric Intake (kJ · d^{-1}, days 1-210)	Treatment (days 211-270)	Body Weight (g, day 211)	Final Body Weight (g, days 211-270)	Caloric Intake (kJ · d^{-1}, days 211-270)
CD (n = 8)	185.0 ± 7.0[a]	414.0 ± 12.0[a]	299.0 ± 13.0[a]	None	414.0 ± 12.0[a]	430.0 ± 11.0[a]	297.0 ± 12.0[a]
SRD (n = 24)	188.0 ± 6.0[a]	470.0 ± 11.0[b]	360.0 ± 9.0[b]	None	472.3 ± 9.0[b]	483.0 ± 9.1[b]	358.0 ± 10.0[b]
				Troglitazone	468.5 ± 10.0[b]	485.4 ± 8.3[b]	357.0 ± 11.0[b]

NOTE. Values are the mean ± SEM. Figures in parentheses indicate the number of individual determinations in each group. Values in each column that do not share the same superscript letter were significantly different ($P < .05$) when 1 variable at a time was compared by the Newman-Keuls test.

5% CO_2: 95% O_2), at a flow rate of 0.9 to 1.2 mL/min.[20] After a prewash period of 30 minutes, two basal samples were obtained. Then, the KRB buffer containing a high glucose concentration (16.8 mmol/L) was used until the end of the perifusion period (40 minutes). Aliquots from the effluent were collected at 1-minute intervals until minute 15, and afterward at 5-minute intervals till minute 40. Samples were stored at −20°C until insulin analysis. The insulin assay sensitivity was 0.5 μU/mL and the intraassay coefficient of variation (CV) was 8.7%, 6.2%, and 5.1% for 1 to 5, 5 to 10, and 10 to 50 μU/mL insulin determination ranges, respectively; the interassay CV was 6.6%, 5.0%, and 5.2% for the given ranges.[15]

Statistical Methods

The results are expressed as the mean ± SEM. The statistical significance of differences between groups was determined by 1-way ANOVA with diet as the main effect, followed by the Newman-Keuls test. To evaluate insulin secretion in the perifusion of isolated islets, 2-tailed Student's *t* test for unpaired samples was used. Differences with a *P* value less than .05 were considered statistically significant.[21]

RESULTS

The increase in weight (15%) and caloric intake observed in rats fed chronically up to 210 days on a SRD[7] were still present when the SRD was administered up to 270 days. Moreover, at the end of the experimental period, these parameters were similarly higher in rats fed either a SRD or SRD + troglitazone as compared with age-matched controls fed the CD for the same period (Table 1). Besides, the relative epididymal fat weight was similarly higher in the group of rats fed either the SRD or SRD + troglitazone as compared with rats fed a CD (CD *v* SRD and SRD + troglitazone, mean ± SEM, n = 8, (g · 100 g body weight, 1.37 ± 0.12 *v* 1.95 ± 0.11, $P < .05$, and 2.10 ± 0.14, $P < .05$).

The relative liver weight (g · 100 g body weight^{-1}), liver protein content (mg · g wet weight^{-1}), and liver marker enzymes AST and ALT (U/L) recorded at the end of the experimental period were similar in all groups (mean ± SEM, n = 8); liver weight: CD, 3.36 ± 0.08; SRD, 3.28 ± 0.06; SRD + troglitazone, 3.31 ± 0.09; liver protein: CD, 255 ± 9; 260 ± 8; SRD + troglitazone, 258 ± 10; AST: CD, 16.5 ± 0.9; SRD, 17.0 ± 2.0; SRD + troglitazone, 19.0 ± 1.6; ALT: CD, 18.5 ± 1.4; SRD, 18.0 ± 1.0; SRD + troglitazone, 18.2 ± 2.5).

According to a previous report from our laboratory,[7] and confirmed by the present findings, plasma FFA and trigyceride levels and liver triglyceride content were significantly higher in rats fed on a SRD for 210 days versus their respective age-matched control rats fed on a CD. Similar changes were observed when the intake of the SRD was prolonged up to 270 days (Table 2). A complete normalization of all of these parameters was recorded in rats fed the SRD when troglitazone was added to the diet from days 210 to 270.

To gain further insight into the possible mechanisms involved in the hypertriglyceridemia in rats chronically fed a SRD, we evaluated ''in vivo'' the triglyceride (VLDL-TG) secretion rate from the liver and the fractional removal rate of the IV fat emulsion from the circulation. Table 3 shows that rats fed the SRD for 270 days had an enlarged plasma triglyceride pool size accompanied by both an increase of plasma triglyceride secretion and a decrease of the fractional removal rates (K_2, percent per minute) compared with age-matched control rats fed the CD. The rats fed with the SRD to which the hypoglycemic agent troglitazone was added from days 210 to 270 showed a complete normalization of all of the parameters.

The rats fed the SRD for 210 days showed a significant increase in basal glucose. Similar changes were recorded when the intake of the SRD was prolonged up to 270 days (Table 4). The addition of the hypoglycemic agent troglitazone to the SRD from days 210 to 270 led to a normalization of plasma basal glucose levels. However, no statistical differences in the plasma insulin concentration were observed at the end of the experimental periods among the different dietary groups.

To assess the effect of a long-term SRD on peripheral insulin sensitivity (insulin resistance), euglycemic-hyperinsulinemic clamp studies were performed at the end of the experimental period. Blood glucose was clamped at 5.5 to 6.0 mmol/L. Values for 5-hour postprandial blood glucose before the clamp were as follows: (mean ± SEM, mmol/L, n = 6): CD, 5.61 ± 0.18; SRD, 7.81 ± 0.15; and SRD + troglitazone, 5.68 ± 0.13. These results were associated with no changes in plasma insulin (μU/mL, n = 6: CD, 39.0 ± 5.5; SRD, 40.0 ± 3.8;

Table 2. Plasma Triglyceride and FFA Concentration and Liver Triglyceride Content in Rats Fed on the Different Diets

Diet	Plasma		Liver
	Triglyceride (mmol/L)	FFA (μmol/L)	Triglyceride (μmol · liver^{-1})
CD (days 1-270)	0.47 ± 0.07[a]	274.1 ± 42.0[a]	175.6 ± 8.6[a]
SRD (days 1-210)	1.70 ± 0.19[b]	685.5 ± 40.3[b]	330.7 ± 40.5[b]
SRD (days 1-270)	1.78 ± 0.20[b]	717.6 ± 54.2[b]	312.3 ± 33.5[b]
SRD (days 1-270) + troglitazone (days 211-270)	0.50 ± 0.04[a]	293.5 ± 22.2[a]	166.8 ± 15.3[a]

NOTE. Values are the mean ± SEM. Six animals were used in each group. Values in each column that do not share the same superscript letter were significantly different ($P < .05$) when 1 variable at a time was compared by the Newman-Keuls test.

Table 3. Plasma TGSR, Plasma Triglyceride Pool Size, and Fractional Removal Rate (K_2, % · min^{-1}) of a Fat Emulsion (Intralipid) in Rats Fed on the Different Diets

Diet	TGSR (nmol · min^{-1} · 100 g body weight^{-1})	TG Pool Size (nmol · 100 g body weight^{-1})	K_2 (% · min^{-1})
CD (days 1-270)	163.0 ± 8.9[a]	1803 ± 102[a]	8.60 ± 1.3[a]
SRD (days 1-270)	212.3 ± 5.8[b]	3410 ± 210[b]	3.01 ± 0.8[b]
SRD (days 1-270) + troglitazone (days 211-270)	165.4 ± 7.5[a]	2000 ± 110[a]	8.04 ± 0.4[a]

NOTE. Values are the mean ± SEM. At least 6 animals were used in each group. Values in each column that do not share the same superscript letter were significantly different ($P < .05$) when 1 variable at a time was compared by the Newman-Keuls test.

SRD + troglitazone, 37.0 ± 4.9). The animals fed on the SRD up to 270 days showed a significant ($P < .01$) decrease of the GIR—which measures insulin action in vivo—compared with rats fed the CD for the same period. On the other hand, when troglitazone was added to the SRD from days 210 to 270, the GIR reached a value similar to that observed in rats fed on the CD (Fig 1). The steady-state of blood glucose and insulin concentrations measured over the last 60 minutes of the clamp are illustrated in the table included in Fig 1. No differences in hematocrit values were observed either within or between groups from the beginning to the end of the clamp.

Figure 2 depicts insulin secretion from the perifused islets of each experimental group. The control group showed the classic biphasic pattern with a first phase (peak) lasting from minute 3 to minute 7 followed by a second phase from minute 10 to minute 40 of perifusion. Perifused islets from SRD-fed rats showed a clear alteration of the biphasic pattern of glucose-stimulated hormone secretion. The first peak of insulin secretion was not observed; however, although insulin levels increased steadily, the values recorded were still significantly lower than the controls. On the other hand, the second phase of the hormone secretion showed values that were significantly greater than the controls. When troglitazone was added to the SRD (from days 211 to 270), the glucose-induced insulin secretion pattern was similar to that in the control group fed on the CD.

DISCUSSION

The present study provides new information on the effects of troglitazone on a nongenetic model of stable dyslipidemia and insulin insensitivity induced in normal rats by feeding with a SRD for a long period.

The major new findings of this study are the following: (1) Triglitazone markedly reduces the in vivo hepatic triglyceride secretion rate and enhances its removal from the circulation, leading to a normalization of plasma triglyceride levels. The agent also normalizes the whole-body peripheral insulin resistance, the glucose homeostasis, and the elevated plasma FFAs without detectable changes in plasma insulin. (2) The clear alteration of the biphasic pattern of glucose-stimulated insulin secretion in the in vitro perifused β-cell islets of rats fed a SRD for a long period is also completely normalized when the SRD is supplemented with troglitazone for 2 months.

The increased triglyceride pool size recorded in rats chronically fed a SRD was accompanied by both a significant increase of the TGSR and a lower fractional removal rate of the fat emulsion (K_2). The latter exhibited a significant correlation in vivo with the fractional turnover rate of endogenous triglyceride.[22] Furthermore, the sustained hypertriglyceridemia with a high liver triglyceride content suggests that a combination of an increase in hepatic fatty acid synthesis and esterification and a defective removal mechanism of triglyceride from the circulation operate in vivo in these animals. When troglitazone was added to the SRD during the last 2 months of the experimental period, it significantly reduced the triglyceride pool size. This is a consequence of both a decrease of the liver TGSR and its enhanced removal from the circulation. Moreover, the sustained decrease of plasma FFAs and liver triglyceride content observed in SRD-fed rats after administration of the agent suggests that a combined effect of reduced plasma FFA availability and esterification may contribute to the normalization of in vivo hepatic triglyceride production and plasma triglyceride levels. In agreement with our results, Fujiwara et al[23] showed that the attenuation of hyperlipidemia (decreased VLDL-TG levels) in C57BL/6J-ob/ob mice and Zucker fatty rats by troglitazone results from an inhibition of triglyceride synthesis in the liver, as well as an increase in clearance from the circulation. Moreover, it has been shown that troglitazone decreases circulating FFA levels in diabetic animal models[9] and obese patients.[24]

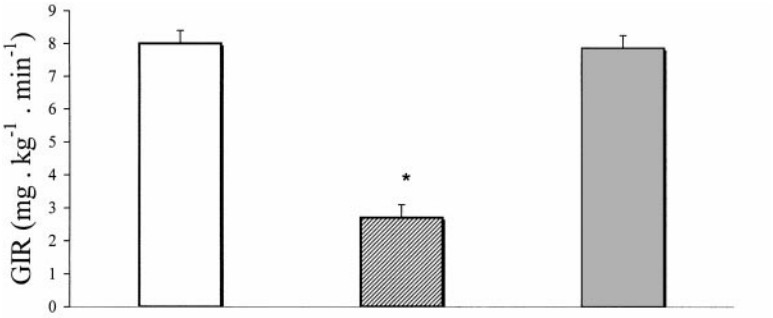
Our results do not provide data concerning the mechanism underlying the antilipolytic effect of the agent. However, some clues have been provided by recent publications. For instance, it has been shown that troglitazone may change the distribution of fat cell size, favoring smaller fat cells that may be more responsive to the antilipolytic actions of insulin.[25] Besides, Oakes et al[26] showed that the thiazolidinedione BRL-49653 has a primary action in adipose tissue to maintain lipid stores by reducing the systemic lipid supply and its subsequent utilization.

On the other hand, the impaired glucose homeostasis and whole-body peripheral insulin resistance were completely normalized at the end of the experimental period when troglitazone was added to the SRD for 2 months. This occurred despite the fact that there were no changes in plasma insulin levels. The rats fed on the SRD to which the agent was added reached a metabolic and hormonal profile resembling that of the age-matched control rats fed a CD. These findings suggest that

Table 4. Plasma Glucose and Insulin in Rats Fed on the Different Diets

Diet	Glucose (mmol/L)	Insulin (μU/mL)
CD (days 1-270)	6.43 ± 0.33[a]	37.6 ± 6.5[a]
SRD (days 1-210)	8.15 ± 0.13[b]	45.7 ± 5.2[a]
SRD (days 1-270)	8.20 ± 0.22[b]	37.1 ± 4.9[a]
SRD (days 1-270) + troglitazone (days 211-270)	6.30 ± 0.22[a]	41.7 ± 5.5[a]

NOTE. Samples for glucose and insulin analysis were taken 1 to 2 hours after food removal. Values are the mean ± SEM. At least 6 animals were used in each group. Values in each column that do not share the same superscript letter were significantly different ($P < .05$) when 1 variable at a time was compared by the Newman-Keuls test.



	CD	SRD	SRD + Troglitazone
Glucose (mmol/L)	5.85±0.12	5.79±0.18	5.93±0.09
Insulin (μU/mL)	735±45	755±38	720±43

Fig 1. GIR during euglycemic-hyperinsulinemic clamp in rats fed on the different diets. □, CD (1-270 days); ▨, SRD (1-270 days); ▩, SRD + troglitazone (SRD days 1-270 + troglitazone days 211-270). Values are the mean ± SEM. Six animals were used in each group. *$P < .05$, SRD *v* CD and SRD *v* SRD + troglitazone. Glucose and insulin, blood glucose and steady-state insulin, respectively, during the last 60 minutes of the clamp.

troglitazone can improve hyperglycemia and insulin sensitivity in either the absence or the presence of a hyperinsulinemic state. The latter was observed in a hyperinsulinemic obese insulin resistance model.[23] Moreover, in vitro investigations have confirmed that the agent affects the skeletal muscle, liver,[27] and adipose tissue,[28] most probably involving both a potentiation of insulin action[9] and a direct insulin-mimicking effect.[27] It has also been reported that the mechanism underlying the improvement of insulin resistance with troglitazone administration includes the stimulation of insulin receptor kinase[29] and GLUT4 translocation.[30]

We observed that SRD-fed rats showed an absence of the first phase and a hypersecretory second phase of insulin secretion in response to a glucose stimulus, as compared with age-matched controls fed a CD. These results are similar to those observed in a previous temporal study between ages 7 and 90 days in the C57BL/KsJ.mbd mouse.[20] Troglitazone treatment in SRD-fed rats restores the normal biphasic insulin secretion similar to that observed in CD-fed rats. In this regard, it has recently been shown that troglitazone exerts a direct lipogenic activity in the islets of obese prediabetic Zucker diabetic fatty rats, and this is related to an increment of glucose- and glucose-arginine–stimulated insulin secretion.[31]

On the other hand, it has also been shown that reduced plasma FFA concentrations by acipimox improve not only insulin-mediated glucose uptake but also the acute insulin response in first-degree relatives of type 2 diabetic patients.[32] Thus, in our studies, the reduction of hypertriglyceridemia and

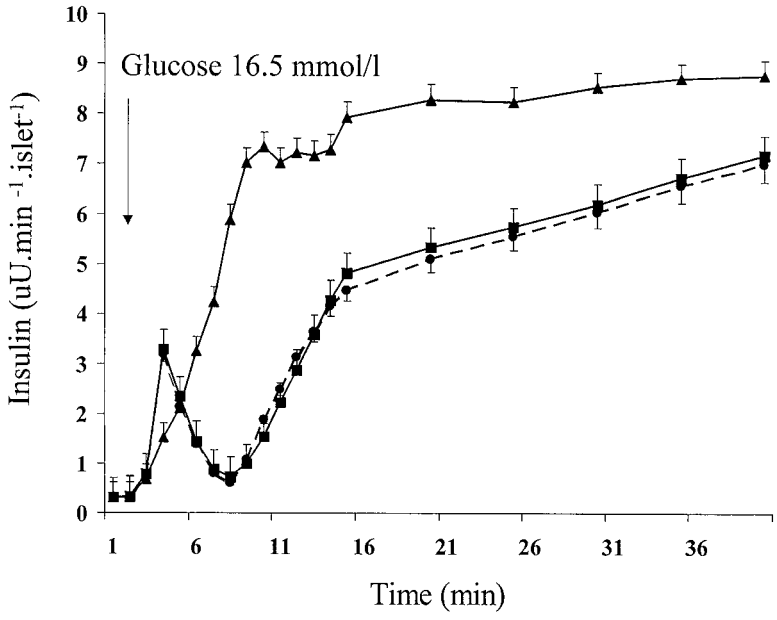


Fig 2. Insulin secretion patterns in perifused pancreatic islets from rats fed on the different diets. ●, CD (1-270 days); ▲, SRD (1-270 days); ■, SRD (1-270 days) + troglitazone (211-270 days). Glucose stimulus (16.5 mmol/L) was added to the perifusion buffer from minutes 3 to 40. Values are the mean ± SEM. Five animals were used in each group. $P < .05$, SRD *v* CD and SRD + troglitazone at minute 4 and from minutes 6 to 40.

fatty acids to normal levels by troglitazone in SRD-fed rats might have a role in the normalization of the insulin secretion pattern, the glucose homeostasis, and the peripheral insulin resistance.

In summary, the current study provides new information regarding the effect of troglitazone on glucose homeostasis and lipid metabolism in an experimental model of dyslipidemia and insulin resistance induced by feeding normal rats with a SRD long-term. The normalization of the altered patterns of glucose-stimulated insulin secretion, as well as the enhancement of peripheral insulin sensitivity, without detectable changes in plasma insulin may be largely a result of the significant action of troglitazone in decreased circulating lipid and enhanced whole-body glucose metabolism.

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