Effect of Troglitazone on Vascular and Glucose Metabolic Actions of Insulin in High–Sucrose-Fed Rats

Marta Santuré, Maryse Pitre, André Nadeau, and Hélène Bachelard

In rats, diets high in simple sugar induce insulin resistance and alter vascular reactivity. The present study was designed to evaluate the effects of 5 weeks treatment with troglitazone on insulin sensitivity, regional hemodynamics, and vascular responses to insulin in chow-fed and high–sucrose-fed rats. Male rats were randomly divided in 4 groups to receive a regular chow diet in the absence (group 1) or presence of troglitazone (0.2% in food; group 2), or a sucrose-enriched diet in the absence (group 3) or presence of troglitazone (group 4) for 5 weeks. The rats were instrumented with Doppler flow probes and intravascular catheters to determine blood pressure, heart rate, and regional blood flows. Insulin sensitivity was assessed by the euglycemic hyperinsulinemic clamp technique. Glucose transport activity was examined in isolated muscles. Sucrose feeding was found to induce insulin resistance and to impair the insulin-mediated skeletal muscle vasodilation. Treatment with troglitazone was found to increase whole-body insulin sensitivity in sucrose- and chow-fed rats, but had no effect on skeletal muscle glucose transport activity measured in isolated muscles from both dietary groups. Changes in regional hemodynamics were observed in both dietary cohorts treated with troglitazone, and the hindquarter vasoconstrictor response to insulin noted in sucrose-fed rats was abolished by the treatment. The vascular effects of troglitazone, and its insulin-related attenuating effects on contractile tone, could have contributed, in part, to improve insulin action on peripheral glucose disposal, presumably by improving blood flow distribution and glucose delivery.
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THE THIAZOLIDINEDIONES represent a new class of oral hypoglycemic agents that appear to work by either mimicking or enhancing insulin action without any effects on β-cell insulin secretion.[1-3] Although the precise mechanism of action of thiazolidinedione compounds, such as troglitazone, ciglitazone, and pioglitazone, has not been fully elucidated, the end results of their physiologic effects are to improve insulin-mediated peripheral glucose disposal and reduce hepatic glucose output, as demonstrated by in vitro and in vivo studies.[3-6] Thus, in diabetic patients and obese subjects, troglitazone proved to be effective in improving peripheral glucose utilization, reducing hepatic glucose output, lowering insulinemia, and reversing dyslipidemia.[4,6,7] The efficacy of troglitazone in improving insulin sensitivity, supressing hepatic gluconeogenesis, and lowering hyperinsulinemia and hypertriglyceridemia has also been reported in various rodent models of insulin resistance.[3,8-10] Interestingly, in addition to their glucose and insulin-lowering effects, thiazolidinediones have been reported to lower blood pressure in insulin-resistant humans, monkeys, and rats.[4,11-16] Thus, treatment with troglitazone and other thiazolidinedione derivatives has been reported to decrease blood pressure in the obese Zucker rats,[16] the Sprague-Dawley rats fed a high-carbohydrate diet,[12,17] the Dahl salt-sensitive rats,[18] and the spontaneously hypertensive rats,[19] which represent both genetic and nongenetic animal models of insulin resistance. Similar hypotensive effects have also been observed in obese subjects with or without impaired glucose tolerance and in patients with type 2 diabetes treated for a few weeks with troglitazone.[4,7] Additionally, it has been reported that patients with type 2 diabetes treated with troglitazone benefited from enhanced cardiac output and stroke volume, possibly as a result of decreased peripheral resistance.[20]

Although the mechanisms underlying the blood pressure-lowering effect of these agents have not been clearly established, several recent findings raise the possibility that these agents may produce vascular and cellular actions that lower blood pressure independently of their ability to increase insulin sensitivity. Indeed, thiazolidinediones have several effects on vascular smooth muscle that may decrease peripheral resistance including (1) inhibition of mitogen stimulated cell growth,[18] (2)attenuation of agonist-mediated calcium uptake,[21] and (3) alteration of vascular response to vasoconstrictor and vasodilator agents.[22] In a recent study, pioglitazone was shown to sensitize aortic tissue isolated from normal rats to an attenuating action of insulin on contractions induced by norepinephrine.[22] In the same study, in vitro incubation with insulin plus pioglitazone was found to enhance acetylcholine-induced, but not nitroprusside-induced vasodilation.[22] Together, these studies raise the possibility that part of the blood pressure-lowering effect of thiazolidinediones might result from direct vascular effect of the drugs, as well as from an insulin-sensitization on vascular function.[11,22] Given that insulin has been reported to increase skeletal muscle blood flow and decrease vascular resistance in insulin-sensitive, but not insulin-resistant subjects[23-25] and given that these effects have been proposed as an important determinant of insulin action on glucose metabolism,[26] it appears to us very important to further examine the effect of a chronic treatment with an insulin-sensitizing agent and conduct experiments looking in parallel at vascular and metabolic actions of insulin.

In a recent study, we found that sucrose feeding in the rat induces insulin resistance and significant alteration in the vascular responses to insulin. The insulin-mediated skeletal muscle

From the Hypertension Research Unit and Diabetes Research Unit, Laval University Hospital Research Center, Ste-Foy, Quebec, Canada.
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Address reprint requests to Hélène Bachelard, PhD, Hypertension Research Unit, Laval University Hospital Research Center, CHUQ, 2705 Blvd Laurier, Ste-Foy, Quebec, Canada, G1V 4G2.

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vasodilation was impaired in sucrose-fed rats.[27] In continuity with our previous study, we undertook a new series of experiments to investigate the effect of long-term treatment (5 weeks) with troglitazone on regional blood flow, insulin sensitivity, and vascular responses to insulin in high–sucrose-fed Sprague-Dawley rats.

MATERIALS AND METHODS

Animals and Feeding Protocol

All surgical and experimental procedures followed institutional animal care guidelines. Fifty-four male Sprague-Dawley rats (Charles River, St-Constant, Canada), aged 5 weeks and initially weighing 200 to 250 g, were housed individually in stainless steel cages. They were placed in a temperature-controlled room (22°C ± 1°C) on a 12 hour/12 hour light/dark cycle (lights on at 6 AM) and had free access to tap water. The animals were randomly divided into 4 groups. Two groups of rats were fed with standard laboratory rat chow (rodent chow 5075, Charles River) in the absence (n = 20) or presence of troglitazone (n = 10; a generous gift from Park Davis, Ann Arbor, MI), and 2 further groups of rats were fed a purified high-sucrose diet in the absence (n = 16) or presence of troglitazone (n = 8) for 5 weeks. Troglitazone was given as a food admixture at a concentration of 0.2%. The high-sucrose diet consisted of 62.5% (wt/wt) sucrose, 6.5% corn oil, 20% protein (casein, purified high nitrogen ; ICN Biochemicals, Montreal, Canada), 0.3% dl-methionine, 1% vitamin mixture (No. 40060; Teklad, Madison, WI), 4.7% mineral mixture (AIN-76 mineral mix, ICN Biochemicals), and 5% cellulose (Alphacel, ICN Biochemicals). The energy density of the diet was 16.81 kJ/g. The animals were allowed to acclimate to their environmental conditions and diets for 3 weeks before the experiments were initiated. During this time the animals had free access to the diet. Body weight and food intake were recorded every other day.

Surgical Preparation

At the end of the acclimation period, the rats from each groups were anesthetized with a mixture of ketamine-xylazine (100 and 10 mg/kg, respectively, intraperitoneally [IP]) and had pulsed Doppler flow probes implanted to monitor changes in renal, mesenteric, and hindquarter blood flows, according to the method previously developed by Gardiner and Bennett[28] and as previously described.[29] The rats were given intramuscular (IM) injections of ampicillin (150 mg/kg) and buprenorphine (0.1 mg/kg) and returned to their home cages. The chow or sucrose diet and the treatment with troglitazone (as applicable) continued during postsurgical recovery, and the latter was deemed satisfactory by the resumption of growth and normalization of 24-hour food intake. At least 7 days later, the rats were reanesthetized with a mixture of ketamine-xylazine (100 and 10 mg/kg, respectively, IP.). The leads of the implanted probes were soldered to a microconnector (Microtech, Boothwyn, PA), and 2 separate catheters were implanted in the right jugular vein (for glucose and insulin infusions) and 1 catheter in the distal abdominal aorta via the left femoral artery (for measurement of blood pressure and heart rate). The catheters were tunneled subcutaneously to emerge at the same point as the probes wires. The rats were given subcutaneous injections of ampicillin (150 mg/kg) and buprenorphine (0.1 mg/kg) and returned to their home cages. The chow or sucrose diet and the treatment with troglitazone (as applicable) continued during this second postsurgical recovery. Experiments began at least 72 hours after this last surgical step in conscious, unrestrained animals with free access to water, but not food. Throughout the experiments, continuous recordings were made of phasic and mean blood pressures, instantaneous heart rate, and phasic and mean renal, superior mesenteric and hindquarter Doppler shift signals using a modified[30] pulsed Doppler monitoring system (Crystal Biotech, Holliston, MA) and a Biopac Data Acquisition and Analysis system (model MP 100; Acknowledge software version 3.1, Goleta, CA). At selected time points (average over 20 seconds), heart rate, mean blood pressure, and mean Doppler shifts were measured and related to the preclamp baseline values (absolute changes for the former 2 variables, percentages for Doppler shifts). In addition, the mean Doppler shift and corresponding mean arterial blood pressure signals were used to calculate percentage changes in regional vascular conductance.

Euglycemic Hyperinsulinemic Clamp Studies

The rats were deprived of food for 12 to 14 hours before the glucose clamp study. Before each experiment, blood glucose and plasma insulin were determined and the resting heart rate, blood pressure, and regional blood flows were recorded over 30 minutes in the quiet, unrestrained, and unsedated rats. Both untreated dietary groups of rats were divided into 2 subgroups. The first subgroup of untreated chow-fed rats (n = 10) and sucrose-fed rats (n = 8), as well as the troglitazone-treated chow-fed group (n = 10) and sucrose-fed group (n = 8), received a continuous infusion of regular porcine insulin (Iletin II ; 100 U/mL, Eli Lilly, Indianapolis, IN) at a rate of 16 mU · kg^{-1} · min^{-1}. Ten minutes after the insulin infusion started, a 50% dextrose solution (made up with saline) was infused at a variable rate to maintain blood glucose at the baseline level (ie, the preclamp level) according to frequent arterial blood glucose determinations performed at 10-minute intervals (Glucometer Elite, Bayer, Etobicoke, Canada). In control experiments, the second subgroup of untreated chow-fed rats (n = 10) and sucrose-fed rats (n = 8) rats was infused with saline-0.2% bovin serum albumin (BSA) instead of insulin and dextrose to match approximately the saline load delivered during the clamp studies. The euglycemic hyperinsulinemic clamp was then performed over 2 hours, while blood pressure, heart rate, and regional blood flow were measured continuously as previously described.[29,31] Continuous recordings of cardiovascular variables were made, but for simplification, only effects measured at the peak responses are presented. The amount of glucose required to maintain euglycemia during the last hour of the clamp, which corresponds to the steady-state concentration of insulin, was used as an index of insulin sensitivity. At the end of the clamp, food (chow or sucrose diets with or without troglitazone) was returned to the rats. Two days later, the rats were deprived again of food for 12 hours, and new experiments were performed to determine glucose transport activity in isolated muscles.

Glucose Transport Activity in Isolated Rat Skeletal Muscles

Basal and insulin-stimulated glucose utilization were examined in isolated soleus and extensor digitorum longus (EDL) skeletal muscles from overnight fasted untreated chow-fed rats (n = 5) and sucrose-fed rats (n = 5) and troglitazone-treated chow-fed rats (n = 5) and sucrose-fed rats (n = 6). Glucose transport in isolated muscles was measured by use of the glucose analogue [^{3}H]-2-deoxy-D-glucose as previously described.[29,31] The rats were anesthetized with a mixture of ketamine-xylazine (100 mg and 10 mg/kg, respectively, IP). Soleus and EDL muscles were dissected out and rapidly cut into 20- to 30-mg strips. The animals were then killed by intracardiac injection of ketamine-xylazine. Muscle strips were incubated in a shaking waterbath at 30°C for 30 minutes in 25-mL flasks containing 3.0 mL oxygenated Krebs-Ringer bicarbonate (KRB) buffer supplemented with 8 mmol/L glucose, 32 mmol/L mannitol, and 0.1% BSA (radioimmunoassay [RIA] grade). The flasks were gassed continuously with 95% O_2 to 5% CO_2 throughout the experiment. After the initial incubation, the muscles were incubated for 30 minutes in oxygenated KRB buffer in the absence or presence of insulin (Humulin R) at 4 different concentrations (0.002, 0.02, 0.2, and 2 mU/mL). The muscles were next washed for 10 minutes at 29°C in 3 mL KRB buffer containing 40 mmol/L mannitol and 0.1% BSA. They were then incubated for 20 minutes at

Table 1. Body Weight, Daily Food Intake, and Baseline Values of Heart Rate, Mean Arterial Blood Pressure, and Regional Doppler Shift and Vascular Conductance of High–Sucrose- and Chow-Fed Rats Left Untreated or After 5 Weeks of Treatment With Troglitazone

Characteristics	Chow-Fed (n = 10)	Sucrose-Fed (n = 8)	Chow-Fed Troglitazone (n = 10)	Sucrose-Fed Troglitazone (n = 8)
Initial body weight (g)	209 ± 11	215 ± 9	197 ± 9	215 ± 15
Final body weight (g)	355 ± 7	344 ± 5	331 ± 8*	337 ± 8
ΔBody weight (g)	+146 ± 11	+130 ± 9	+134 ± 6	+122 ± 14
Daily food intake (g)	28 ± 1†	22 ± 1	22 ± 1	21 ± 1
Heart rate (beats/min)	320 ± 10	329 ± 7	306 ± 5	330 ± 5
Mean arterial pressure (mm Hg)	85 ± 2	83 ± 2	82 ± 2	84 ± 3
Doppler shift (kHz)				
Renal	8.7 ± 1.1	7.5 ± 0.6	9.4 ± 0.7	9.5 ± 0.6*
Mesenteric	12.3 ± 1.7	11.5 ± 1.1	18.8 ± 1.7*	13.7 ± 1.2‡
Hindquarter	5.7 ± 0.6	6.7 ± 0.6	9.2 ± 1.9*	8.4 ± 1.2
Vascular conductance (kHz · mm Hg^{-1}) 10^3				
Renal	102 ± 12	90 ± 6	115 ± 9	116 ± 10*
Mesenteric	145 ± 21	137 ± 13	231 ± 22*	167 ± 19‡
Hindquarter	67 ± 6	82 ± 9	110 ± 21*	102 ± 15

NOTE. Values are means ± SE; n is the number of rats. The groups represent those used to assess hemodynamic effects of insulin intravenously infused during the clamp studies.

*$P < .05$ treated chow- or sucrose-fed group *v* respective untreated group.

‡$P < .05$ treated sucrose-fed group *v* treated chow-fed group.

†$P < .05$ untreated chow-fed group *v* the 3 other groups of rats. Student's *t* test for unpaired data.

29°C in 3 mL KRB buffer containing 8 mmol/L [^{3}H]-2-deoxy-D-glucose (2.25 μCi/mL), 32 mmol/L [^{14}C]-mannitol (0.3 μCi/mL), 2 mmol/L sodium pyruvate, and 0.1% BSA. Insulin was present throughout the wash and uptake incubations (if it was present in the previous incubation medium). After the incubation, muscles were rapidly blotted at 4°C, clamp-frozen, and stored at -80°C until processed. Muscles were processed by boiling for 10 minutes in 1 mL water. Extracts were transferred to an ice bath, vortexed, and then centrifuged at 1,000 × *g*. Triplicate 200-μL aliquots of the muscle extract supernatant and of the incubation medium were counted for radioactivity using a Wallac 1409 counter (Perkin Elmer Life Sciences, Boston, MA). [^{3}H]-2-deoxy-D-glucose uptake rates were corrected for extracellular trapping using [^{14}C]-mannitol.[32]

Analytic Methods

Blood samples for plasma glucose and insulin determinations in the basal state and during insulin infusion were obtained, placed in untreated polypropylene tubes, and centrifuged with an Eppendorf microcentrifuge (Minimax; International Equipment, Needham Heights, IL). The plasma was stored at −20°C until assay. The glucose concentration of the supernatant was measured by the glucose oxidase method[33] using a glucose analyzer (Technicon RA-XT, Bayer, Tarrytown, NY), and the plasma insulin level was measured by RIA using porcine insulin standards and polyethylene glycol for separation.[34]

Data Analysis

Values are expressed as means ± SE; n is the number of observations. Data describing the biologic characteristics of the rats were evaluated using Student's *t* test for unpaired data, whereas results obtained over time, such as those from cardiovascular responses to insulin, were analyzed for statistical significance by an analysis of variance (ANOVA) for repeated measurements. Post hoc comparisons were made using Fisher's test. $P < .05$ was considered significant.

RESULTS

Weight and Hemodynamic Changes

Table 1 illustrates the effects of the long-term diets and troglitazone treatment on body weight and resting values for mean arterial blood pressure, heart rate, and regional blood flows and vascular conductances. These results indicate that after the 5 weeks of feeding in the absence or presence of troglitazone rats displayed comparable body weight increases regardless of whether they were fed the high sucrose or the normal chow diet, or whether they were treated or not with troglitazone. Moreover, as shown in Table 1, sucrose feeding was not associated with significant changes in mean arterial blood pressure, heart rate, or regional blood flows or vascular conductances as compared with values measured in the normal chow-fed group. However, in the chow-fed group, 5 weeks of treatment with troglitazone was found to significantly increase superior mesenteric and hindquarter blood flows and vascular conductances as compared with values measured in the untreated chow-fed group. Moreover, there was a tendency toward higher baseline values for renal blood flow and vascular conductance in the troglitazone-treated chow-fed rats than in the untreated chow-fed rats, but this effect did not reach the level of significance. In the sucrose-fed group, the troglitazone treatment was found to significantly increase renal blood flow and vascular conductance as compared with values measured in the untreated sucrose-fed group. Moreover, a tendency toward higher baseline values for superior mesenteric and hindquarter blood flows and vascular conductances was also noted in the troglitazone-treated group when compared with the untreated sucrose-fed group, but these effects did not reach the level of significance.

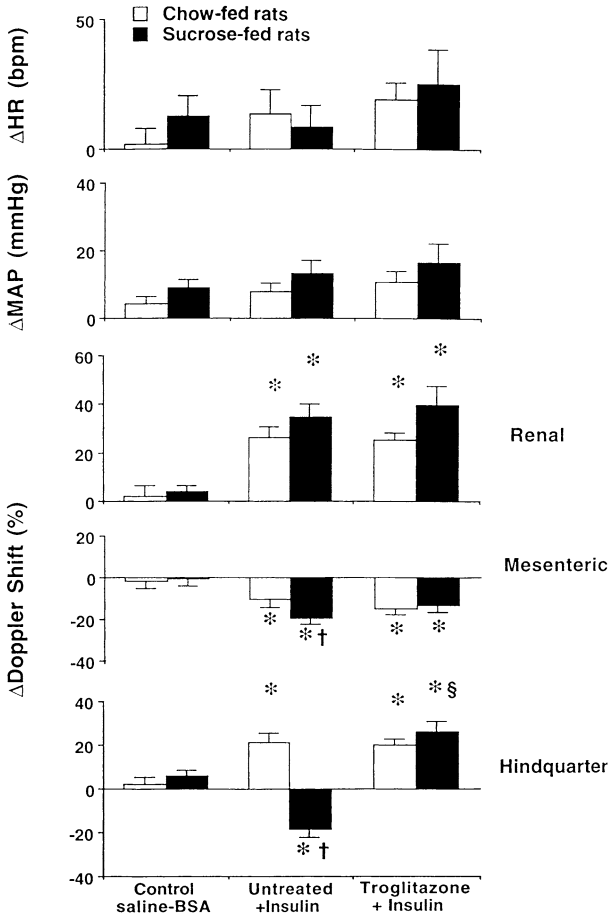

Fig 1. Bar graph illustrating the maximum cardiovascular changes elicited by control intravenous (IV) infusion of saline-0.2% BSA in chow- (n = 10) or sucrose-fed (n = 8) rats or by euglycemic infusion of insulin (16 mU · kg^{-1} · min^{-1}) in chow- (n = 10) or sucrose-fed (n = 8) rats or in troglitazone-treated chow-fed (n = 10) or sucrose-fed (n = 8) rats. Effects of saline-0.2% BSA or insulin were assessed relative to baseline values. Values are means with SE shown by vertical lines. *P < .05 for the insulin-infused groups *v* their respective control saline-BSA groups, ANOVA followed by Fisher's test. †P < .05 for the untreated sucrose-fed group receiving IV infusion of insulin *v* the untreated chow-fed group receiving the same IV infusion of insulin, ANOVA followed by Fisher's test. §P < .05 for the troglitazone-treated sucrose-fed group *v* the untreated sucrose-fed group receiving IV infusion of insulin, ANOVA followed by Fisher's test. MAP, mean arterial blood pressure; HR, heart rate; bpm, beat per minute.

Hemodynamic Responses to Insulin Infusion During the Euglycemic Hyperinsulinemic Clamp Period

Figure 1 shows that insulin infusion at a rate of 16 mU · kg^{-1} · min^{-1} in a group of chow-fed rats produced cardiovascular changes characterized by significant increases in renal (maximum change observed 90 minutes after the beginning of insulin infusion) and hindquarter (maximum change observed at 105 minutes) flows, but no significant changes were seen in heart rate or mean arterial blood pressure (Fig 1). Moreover, there was a slight, but significant, decrease in superior mesenteric flow (maximum at 75 minutes). Furthermore, long-lasting increases were noted in renal (peak response at 90 minutes) and hindquarter (peak response at 105 minutes) vascular conductances, whereas a significant decrease in superior mesenteric vascular conductance (maximum change at 75 minutes) was observed (Fig 2). Similar cardiovascular changes were observed in troglitazone-treated chow-fed rats.

In sucrose-fed rats, the same infusion of insulin had no effect on heart rate or mean arterial blood pressure, while a significant increase in renal flow (maximum change at 90 minutes) was observed when compared with the effects of control infusion of saline-0.2% BSA in sucrose-fed rats (Fig 1). These responses were not different from those seen in chow-fed rats. However,

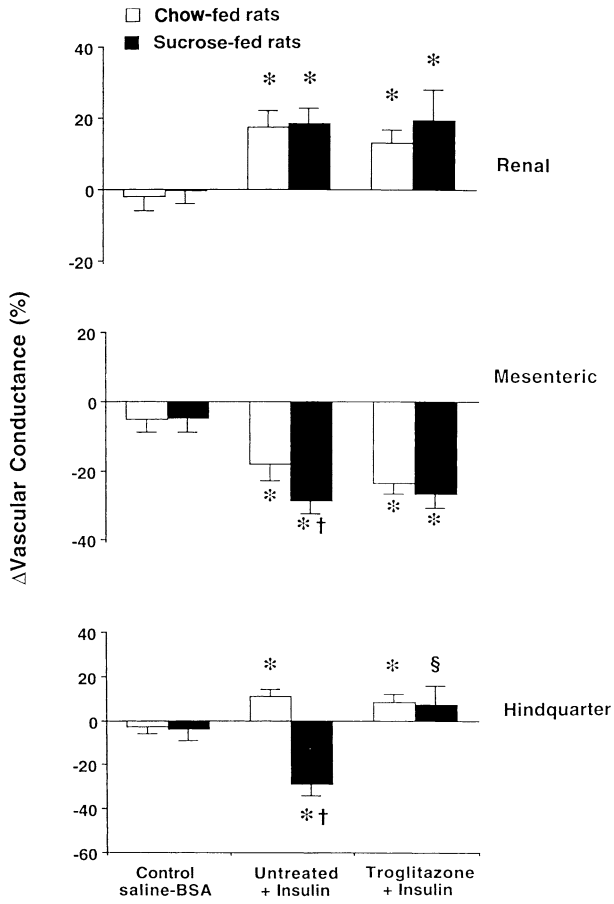

Fig 2. Bar graph illustrating the maximum changes in renal, superior mesenteric and hindquarter vascular conductances elicited by control IV infusion of saline-0.2% BSA in chow- (n = 10) or sucrose-fed (n = 8) rats or by euglycemic infusion of insulin (16 mU · kg^{-1} · min^{-1}) in untreated chow- (n = 10) or sucrose-fed (n = 8) rats or in troglitazone-treated chow-fed (n = 10) or sucrose-fed (n = 8) rats. These data were derived from the data shown in Fig 1. Effects of saline-0.2% BSA or insulin were assessed relative to baseline values. Values are means with SE shown by vertical lines. *P < .05 for the insulin-infused groups *v* their respective control saline-BSA groups, ANOVA followed by Fisher's test. †P < .05 for the untreated sucrose-fed group receiving IV infusion of insulin *v* the untreated chow-fed group receiving the same IV infusion of insulin, ANOVA followed by Fisher's test. §P < .05 for the troglitazone-treated sucrose-fed group *v* the untreated sucrose-fed group receiving IV infusion of insulin, ANOVA followed by Fisher's test.

insulin infusion in sucrose-fed rats caused significant decreases in superior mesenteric (peak response at 90 minutes) and hindquarter (peak response at 60 minutes) flows, which differed significantly from our observations in chow-fed rats (Fig 1). These cardiovascular responses were associated with increases in renal vascular conductance (maximum change at 60 minutes), which was not different from that seen in chow-fed rats, and decreases in superior mesenteric (maximum change at 90 minutes) and hindquarter (maximum change at 105 minutes) vascular conductances, when compared with the effects of control infusion of saline-0.2% BSA in sucrose-fed rats (Fig 2). These latter responses differed significantly from those seen in chow-fed rats, in which insulin infusion at a dose of 16 mU · kg^{-1} · min^{-1} produced a smaller vasoconstriction in the superior mesenteric vascular bed and a vasodilation in the hindquarter, instead of a vasoconstriction. Five weeks treatment with troglitazone in sucrose-fed rats had no effect on renal and superior mesenteric blood flow responses to insulin, but it was found to blunt the reduction in hindquarter flow and replace it by a significant increase in blood flow (peak response at 105 minutes) (Fig 1). Moreover, the hindquarter vasoconstrictor response to insulin was completely abrogated in the troglitazone-treated sucrose-fed rats (Fig 2).

Responses During Euglycemic Hyperinsulinemic Clamp

Figure 3 shows that, in the fasting state, basal arterial blood glucose and plasma insulin levels were similar in the 4 groups of rats studied. During the euglycemic hyperinsulinemic clamp, we found that plasma insulin levels in the 4 groups of rats increased acutely and achieved similar plateaus, whereas normal blood glucose levels were maintained in every group of rats. However, the average glucose infusion rate required to maintain euglycemia during the last hour of the clamp ($GIR_{60\text{-}120}$), the conditions of which closely approximated a steady-state insulin concentration and which represented the whole-body glucose utilization, were significantly smaller in the sucrose-fed rats than in the chow-fed control group. Five weeks treatment with troglitazone in chow-fed rats and sucrose-fed rats was found to significantly increase the index of insulin sensitivity ($GIR_{60\text{-}120}$) when compared with their respective dietary control group.

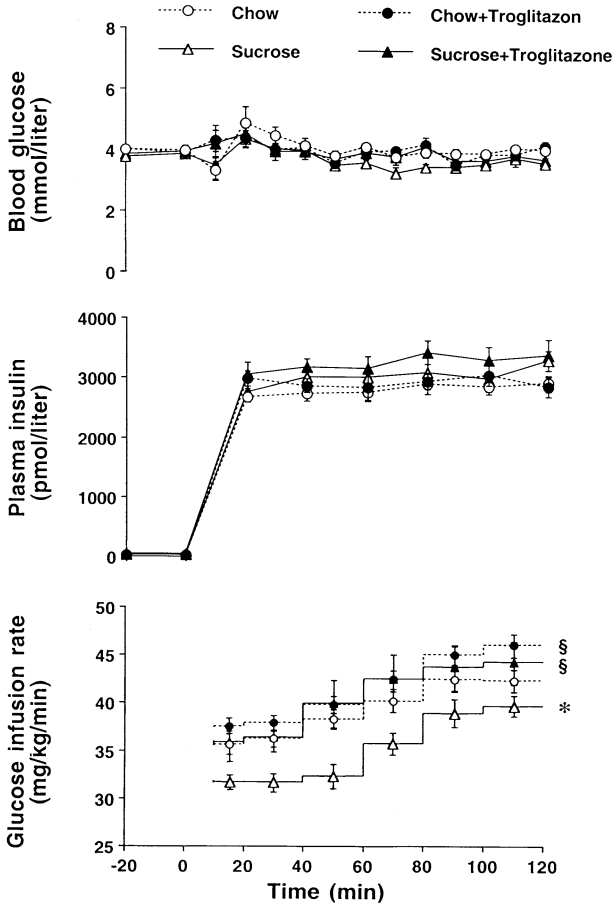

Fig 3. Summary of steady-state blood glucose and plasma insulin concentrations and glucose infusion rate during a euglycemic hyperinsulinemic clamp performed in untreated chow-fed (n = 10) and sucrose-fed rats (n = 8) and troglitazone-treated chow-fed (n = 10) and sucrose-fed (n = 8) rats. The insulin infusion rate used in that study was 16 mU · kg^{-1} · min^{-1}. Data are presented as means ± SE shown by vertical lines. *$P < .05$ for the untreated sucrose-fed rats *v* the untreated chow-fed rats, ANOVA followed by Fisher's test. §$P < .05$ for both groups of troglitazone-treated rats *v* their respective untreated control group, ANOVA followed by Fisher's test.

Effect of High-Sucrose Diet on [^{3}H]-2-Deoxy-D-Glucose Uptake in Isolated Skeletal Muscles

The effect of the long-term diets and treatment with troglitazone on basal and insulin-stimulated glucose uptake in isolated soleus and EDL muscles is shown in Fig 4. In both isolated muscles, we found that the sucrose-enriched diet had no influence on basal glucose transport activity compared with that observed in chow-fed rats. However, in the presence of low doses of insulin (ie, 0.002 and 0.02 mU/mL), we found a significant reduction in insulin-activated glucose transport compared with that measured in chow-fed rats. These differences were no longer observed in the presence of higher doses of insulin (ie, 0.2 and 2 mU/mL). Moreover, as shown in Fig 4, we found that 5 weeks of treatment with troglitazone in both dietary cohorts had no influence on basal or insulin-stimulated glucose transport activity (at any doses of insulin tested) in isolated soleus and EDL muscles when compared with that measured in their respective untreated control group.

DISCUSSION

The demonstration in this study that sucrose feeding produces a reduction in whole-body insulin sensitivity in the sucrose-fed rats when compared with the chow-fed rats is consistent with previous studies performed in rats and indicating the capacity of diets high in simple sugars to reduce insulin sensitivity.[35,36] The present study indicates that 5 weeks of treatment with troglitazone significantly improved insulin sensitivity in high–sucrose-fed rats, as determined using the euglycemic hyperinsulinemic clamp technique. Moreover, a slight, but significant, increase in insulin sensitivity index was also noted in chow-fed rats treated with troglitazone when compared with the untreated chow-fed group. The thiazo-

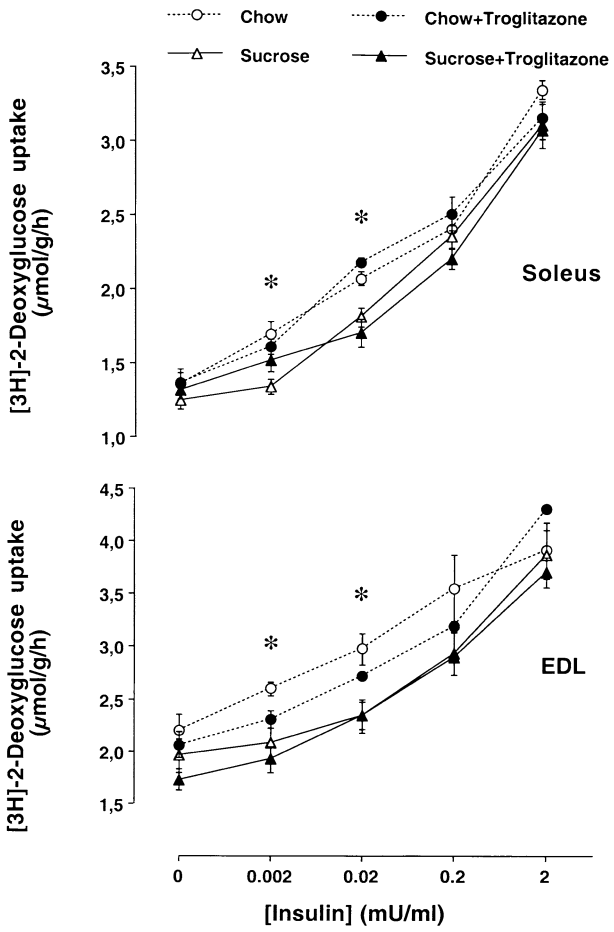

Fig 4. Insulin dose-response curve for stimulation of glucose uptake in soleus muscle and EDL muscle. Muscles were dissected out from chow-fed rats (n = 5), sucrose-fed rats (n = 5), troglitazone-treated chow-fed rats (n = 5), and troglitazone-treated sucrose-fed rats (n = 6). Values are means ± SE shown by vertical lines. *$P < .05$ for the untreated and troglitazone-treated sucrose-fed rats *v* the untreated and troglitazone-treated chow-fed rats, respectively, Student's *t* test for unpaired data.

lidinediones have been reported to restore the ability of insulin to suppress hepatic glucose output and to increase peripheral glucose disposal in animal models of insulin resistance[8-10,13,37] and in humans with type 2 diabetes, as well as other syndromes characterized by insulin resistance.[4,6,15] These effects may have contributed to increase the insulin sensitivity index measured during the euglycemic hyperinsulinemic clamp performed in both troglitazone-treated groups of rats.

Although the precise mechanism of action of thiazolidinediones has not been fully elucidated, it is possible that part of the beneficial effects of troglitazone on peripheral insulin sensitivity was secondary to troglitazone-induced hemodynamic changes leading to increased tissue perfusion and increased glucose uptake. As seen in the present study, 5 weeks of treatment with troglitazone had no effect on mean arterial blood pressure, but caused significant increases in regional blood flows and vascular conductances in both dietary cohorts. These findings are consistent with those of Fujishima et al[38] indicating that a single oral dose of troglitazone increased forearm vasodilation in healthy humans and those of Fujiwara et al[39] demonstrating that troglitazone treatment increased skin blood flow in diabetic rats and normal rats. Moreover, our results support previous findings showing that troglitazone decreased perfusion pressure in isolated perfused rat hind limb.[39] On a quantitative basis, skeletal muscle has been identified as the predominant site of insulin-stimulated glucose disposal and as the major tissue responsible for postprandial hyperglycemia in insulin-resistant states.[23,40,41] Therefore, it is likely that the hemodynamic changes seen in the present study with the long-term treatment with troglitazone, particularly at the skeletal muscle level, may have contributed to enhance insulin action on glucose metabolism through increased blood flow distribution and then glucose delivery to insulin-sensitive tissues. Furthermore, the hemodynamic changes noted here could also explain, at least in part, the antihypertensive effects frequently reported during chronic administration of thiazolidinedione in a variety of settings characterized by normal and reduced insulin action on glucose metabolism. Thus, a number of studies have demonstrated a blood pressure-lowering effect of various thiazolidinediones in humans with obesity, impaired glucose tolerance, or type 2 diabetes,[4,6,15] as well as in several insulin-resistant rat models, eg, the Dahl-salt sensitive rat, the obese Zucker rat, the Sprague Dawley rats fed high-carbohydrate or high-fat diets.[11,12,14,17,18] The demonstration in some studies that the capacity of thiazolidinediones to reduce blood pressure is not invariably associated with their capacity to increase insulin sensitivity,[42] highly suggests that the hemodynamic effects of thiazolidinediones, rather than their improving effect on insulin sensitivity, are probably the primary mechanism involved in their blood pressure-lowering actions. This is supported by some recent in vitro studies indicating that thiazolidinediones have direct vascular actions possibly through a mechanism related to inhibition of calcium entry into vascular smooth muscle cells (through L-type calcium channels).[11,21] On the other hand, we cannot exclude the possibility that alterations in free fatty acids plasma levels induced by sucrose diet and troglitazone treatment may have contributed to alter vascular endothelial responses and muscle glucose uptake. However, although we did not determine the effect of sucrose diet and troglitazone treatment on free fatty acid metabolism in the present study, we recently published a study performed under the same experimental conditions, demonstrating no significant diet effects on plasma nonesterified fatty acids levels between the 2 dietary cohorts, the chow-fed and sucrose-fed rats.[27]

In apparent contrast with these previous studies, we failed to show any blood pressure-lowering effect of treatment with troglitazone in our sucrose- and chow-fed rats, while significant increases in regional vascular conductances and whole body insulin sensitivity were noted in both dietary groups. A possible explanation could be that the vasodilator effect (depressor) of troglitazone treatment was offset by an opposing effect of troglitazone on cardiac output, as previously shown in patients with type 2 diabetes.[20] Hence, an increased cardiac output with increased total vascular conductance may result in no change in blood pressure. On the other hand, we cannot exclude the possibility that the type and the dose of the thiazolidinedione

derivative used, as well as differences in the severity of insulin resistance and basal level of blood pressure, may have contributed to prevent the antihypertensive effect of troglitazone in our rat models. In this study, using Sprague Dawley rats chronically instrumented with intra-arterial catheters to continuously record direct intra-arterial pressure in quiet and unrestrained rats, we noted no significant diet effect on blood pressure. These results agreed with those of Brands et al,[43] also based on continuous measurements of intra-arterial pressure and indicating that inducing insulin resistance with a high-fructose diet has no effect on blood pressure. Other investigators have also failed to show any hypertensive effect of high sugar intake when blood pressure was measured in a simple intra-arterial recording.[17,44] In contrast, and almost always based on a simple and indirect measurement of tail systolic blood pressure, several reports indicate that feeding rats a diet high in glucose, sucrose, or fructose causes hypertension.[35,45,46] These conflicting results have been attributed to differences in the duration and contents of the diets in the various studies and to variable blood pressure responses to high carbohydrate diet among different ages and strains of rats.[47,48] Moreover, it has been suggested that the conditions of the blood pressure measurement itself may have confounded accurate assessment of blood pressure in these animals.[47,49]

Although no diet effects were noted on resting blood pressure, heart rate, or regional blood flows, the vascular responses to insulin were significantly altered by the high sucrose diet. In untreated chow-fed rats, the euglycemic infusion of insulin elicited vasodilations in renal and hindquarter vascular beds, a slight vasoconstriction in the superior mesenteric vascular bed, but no changes in mean arterial blood pressure or heart rate. These cardiovascular responses are similar to those we previously reported in normal Wistar and Sprague Dawley rats.[31] In high–sucrose-fed rats, using the same infusion of insulin, we found that the insulin's vasodilating action in skeletal muscle vasculature was impaired. This impairment may have contributed to deficient glucose uptake and insulin resistance due to less delivery of glucose to muscle cells. The insulin-mediated skeletal muscle vasodilation, shown to be nitric oxide-dependent,[50] is thought to represent a normal physiologic mechanism that contributes to enhance insulin's overall action on glucose disposal by increasing glucose delivery to insulin-sensitive tissues.[23,24] Consistent with this is the positive correlation reported between insulin-mediated vasodilation and the ability of insulin to mediate glucose uptake in skeletal muscle.[23] Impairment in the insulin-mediated skeletal muscle vasodilator response has been shown in several states of insulin resistance, such as obesity, type 2 diabetes, and hypertension.[23-25] Thus, one of the aims of the present study was to examine concomitantly the effect of long-term treatment with troglitazone on insulin sensitivity and vascular responses to insulin in high–sucrose-fed rats, and their dietary control, the chow-fed rats. Our results indicate that 5 weeks of treatment with troglitazone completely abolished the hindquarter vasoconstrictor response to insulin in sucrose-fed rats, but had no effect on the cardiovascular responses elicited by insulin in chow-fed rats. The present findings support previous in vitro studies indicating that thiazolidinediones affect vascular reactivity and demonstrating the ability of the insulin-sensitizing agent, pioglitazone, to modulate the effect of insulin on vascular function, resulting in both blunted vasoconstriction and augmented endothelium-dependent vasodilation in rat aortic tissues.[11,22] Therefore, we propose that the inhibition of the insulin-mediated hindquarter vasoconstrictor effect noted in our sucrose-fed rats may have contributed to improve glucose distribution to a greater mass of insulin-sensitive tissues and then to enhance the insulin action on glucose metabolism.

Since approximately 80% of overall in vivo insulin-stimulated glucose disposal involves uptake into skeletal muscle, it seems likely that, independently of (or concomitantly with) the proposed hemodynamic mechanism, a potentiation of insulin action on glucose extraction in muscle represents a primary mechanism of the manner in which troglitazone improves insulin sensitivity in sucrose-fed rats. This would be consistent with previous findings indicating that troglitazone is able to potentiate insulin-stimulated glucose uptake in cultured myocytes.[51] The information to date indicate that the ability of various thiazolidinediones to augment glucose transport activity is often associated with enhanced expression of the glucose transporters, GLUT1 and GLUT4, as determined in cultured muscle cells.[8,51] In this study, we sought to evaluate the effect of long-term treatment with troglitazone in vivo on basal and insulin-stimulated glucose transport activity in isolated skeletal muscles, thus in the absence of blood flow influence. The soleus and EDL muscles were obtained from untreated sucrose- and chow-fed rats and from troglitazone-treated sucrose- and chow-fed rats. Our results show that skeletal muscles isolated from untreated sucrose-fed rats were less sensitive to the insulin-stimulating effect on glucose transport when compared with chow-fed rats. Five weeks of treatment with troglitazone was found to have no effect on basal or insulin-stimulated glucose transport activity in both muscles isolated from both dietary cohorts. Although the present findings may emphasize the importance of blood flow in the effect of troglitazone on peripheral glucose disposal, these results are at variance with previous studies indicating that treatment of insulin-resistant rodents with thiazolidinediones augment glucose uptake into muscle. However, the discrepancy may best be explained by differences in the experimental design and procedures. Thus, differences in the group population (eg, the animal species and the rat strain, the severity of insulin resistance), the preparation used (whole animal, isolated muscle, or cultured myocytes), the thiazolidinedione derivative used, the dose used, as well as the duration of the treatment, may have contributed to these seemingly disparate results. Alternatively, we cannot exclude the possibility that the failure to detect any increases in glucose transport activity following treatment with troglitazone resulted from limited assay sensitivity or from a type II statistical error (nonrejection of the null hypothesis when a difference really exists) particularly when comparisons are restricted to a small sample size. Thus, variability in measurement of glucose transport activity, both within and between groups, may have contributed to obscure any significant effect of the treatment with troglitazone.

In summary, the present data indicate that sucrose feeding in the rat induces insulin resistance, but has no effect on blood pressure, heart rate, or regional hemodynamics, as determined in vivo by using the euglycemic hyperinsulinemic clamp tech-

nique and concomitant measurement of continuous blood pressure, heart rate, and regional blood flows and in vitro by measuring glucose transport activity in isolated muscles. Moreover, we noted that the insulin's physiologic effect to vasodilate skeletal muscle was impaired in the sucrose-fed rats. Treatment with troglitazone was found to significantly improve whole body insulin-mediated glucose disposal in sucrose-fed rats. A slight, but significant, increase in insulin sensitivity index was also noted in the chow-fed rats receiving troglitazone. These effects of troglitazone on peripheral glucose disposal were not associated with significant changes in basal or insulin-stimulated glucose transport activity in isolated muscles from both dietary groups. However, significant changes in regional hemodynamics were observed in both dietary cohorts, and the hindquarter vasoconstrictor response to insulin noted in untreated sucrose-fed rats was completely abolished by the treatment with troglitazone. Based on these observations, we suggest that the vascular effects of troglitazone, and its insulin-related attenuating effects on contractile tone, resulting in blunted hindquarter vasoconstrictor response in sucrose-fed rats, may contribute, in part, to improve insulin action on peripheral glucose disposal, presumably by improving blood flow distribution and glucose delivery to insulin-sensitive tissues. According to other animal and human studies, we believe that the present findings could be generalized to other members of the thiazolidinedone class, such as rosiglitazone and pioglitazone, because of the role of peroxisome proliferator-activated receptor (PPAR)-γ in vascular physiology.[22,52-56]

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