The Thiazolidinedione Rosiglitazone (BRL-49653) Lowers Blood Pressure and Protects Against Impairment of Endothelial Function in Zucker Fatty Rats

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Human obesity is associated with insulin resistance, hyperinsulinemia, and a predisposition to hypertension and vascular disease, the origin of which may lie in impairment of endothelial function. We tested the effects of the thiazolidinedione rosiglitazone on blood pressure and endothelial function in insulin-resistant fatty Zucker rats, which display hypertension and abnormal endothelial cell function. We studied fatty Zucker rats given rosiglitazone maleate (50 µmol/kg diet; n = 8) for 9–12 weeks (treated fatty), untreated fatty rats (n = 8), and lean rats (n = 8) given diet alone. At the end of the study, systolic blood pressure was significantly higher in untreated fatty (147 ± 5 mmHg) than in lean rats (125 ± 2 mmHg; $P < 0.05$), but rosiglitazone treatment prevented the development of hypertension in fatty rats (123 ± 1 mmHg). Fasting hyperinsulinemia in untreated fatty rats (28.7 ± 6.0 ng/ml) was significantly lowered by rosiglitazone (7.0 ± 1.4 ng/ml; $P < 0.05$ vs. untreated fatty), but remained significantly higher than the levels seen in lean rats (1.5 ± 0.4 ng/ml; $P < 0.01$). Mesenteric arteries were studied in a myograph. Maximal acetylcholine chloride (1.1 µmol/l)-induced relaxation of norepinephrine hydrochloride (NE)-induced constriction was impaired in untreated fatty (62.4 ± 3.4%) vs. lean (74.3 ± 3.5%; $P = 0.01$) rats; this defect was partially prevented by rosiglitazone (66.5 ± 3.0%; $P = 0.01$ vs. untreated fatty). Insulin (50 mU/l) significantly attenuated the contractile response to NE in lean rats (14.7 ± 3.3%; $P = 0.02$); this vasodilator effect of insulin was absent in untreated fatty rats at concentrations of 50–5,000 mU/l, but was partially restored by rosiglitazone (9.7 ± 2.5% attenuation; $P = 0.02$ vs. no insulin). Thus, rosiglitazone prevents the development of hypertension and partially protects against impaired endothelial function associated with insulin resistance. These latter effects may contribute to the drug's antihypertensive properties. *Diabetes* 48:1448–1453, 1999

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ACh, acetylcholine chloride; ANOVA, analysis of variance; KPSS, physiologic salt solution with K instead of NaCl; NE, norepinephrine hydrochloride; PSS, physiologic salt solution.

The clustering of insulin resistance, abnormal glucose tolerance, arterial hypertension, abdominal obesity, and dyslipidemia known as the metabolic syndrome (syndrome X) (1) is associated with a substantially increased cardiovascular risk (2). It has been argued that insulin resistance may be the primary abnormality in syndrome X (1), but the relationship to hypertension remains unexplained. In addition to its metabolic effects, insulin has other actions that could influence the development of hypertension. Insulin stimulates sympathetic activity (3) and causes renal sodium and water retention (4,5), effects that would predispose to hypertension. Conversely, insulin has a vasodilator action in various vascular beds (3,6–8), apparently due to the stimulation of release of endothelium-derived nitric oxide (8,9), which would tend to lower blood pressure.

Hypertension could theoretically result from insensitivity to insulin's vasodilator action, particularly if sensitivity to its pressor effects were preserved (10). Insulin-induced vasodilation is impaired in insulin-resistant humans (11,12). We have previously demonstrated that endothelial function is abnormal in fatty Zucker rats and, in particular, that insulin's ability to oppose norepinephrine (NE)-induced vasoconstriction is attenuated before the development of hypertension (13).

In many respects, the obese (fatty; *fa/fa*) Zucker rat mimics human syndrome X. It displays resistance to the metabolic actions of insulin, particularly in skeletal muscle (14), together with dyslipidemia, mild glucose intolerance, marked hyperinsulinemia, and in some colonies, hypertension that develops by 4–5 months of age (15). In contrast, their lean counterparts (*Fa/Fa* and *Fa/fa*) are insulin-sensitive and normoinsulinemic, with a normal lipid profile, glucose tolerance, and blood pressure. The fatty Zucker rat is therefore a potentially useful model to study the links between insulin resistance and cardiovascular disease and the mechanisms and benefits of pharmacologic treatment to ameliorate insulin resistance.

The thiazolidinedione insulin-sensitizing drugs are a new and potentially useful development in the treatment of insulin-resistant states, in particular, type 2 diabetes and impaired glucose tolerance. Thiazolidinediones enhance peripheral insulin sensitivity in animal models of insulin resistance (16–18), and troglitazone has been shown to have similar beneficial effects on type 2 diabetes (19) and in obese insulin-resistant subjects who were normoglycemic or had impaired glucose tolerance (20). Their mechanism of action

is not entirely clear. Their binding to the nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ), which is suggested to improve glucose metabolism by decreasing free fatty acid levels, parallels their insulin-sensitizing potency (21). Thiazolidinediones also have other effects, including blocking the action of tumor necrosis factor-α (TNF-α) (22), a cytokine that induces insulin resistance by inhibiting the insulin receptor's tyrosine kinase activity (23). Interestingly, thiazolidinediones lower blood pressure in hypertensive fatty Zucker rats (24,25), obese diabetic rats (26), diet-induced hypertensive rats (27,28), and obese insulin-resistant humans (20). The mechanism by which blood pressure falls is not known.

In this study, we aimed to determine whether the amelioration of metabolic insulin resistance induced by the thiazolidinedione rosiglitazone in fatty Zucker rats was associated with a fall in blood pressure and with improved resistance artery responses to insulin and acetylcholine (ACh) in vitro.

RESEARCH DESIGN AND METHODS

Animals. We studied male Zucker rats obtained from Harlan U.K. (Bicester, Oxon). All animals were housed at an ambient temperature of 22°C, exposed to a 12-h light-dark cycle, had free access to water, and were habituated to frequent handling. Fatty Zucker rats were fed either standard laboratory powdered diet (R & MI, SDS, Cambridgeshire, U.K.) (untreated fatty rats; $n = 8$) or powdered diet containing rosiglitazone maleate (BRL-49653 maleate; SmithKline Beecham Pharmaceuticals, Essex, U.K.) at a concentration of 50 µmol/kg diet, equivalent to a daily dose of 7–7.5 µmol/kg body wt (treated fatty rats; $n = 8$), from age 6 weeks for 3 months; this regimen has been shown to enhance insulin sensitivity in fatty Zucker rats (29). Because rosiglitazone can stimulate feeding and cause weight gain in fatty Zucker rats (30), the rosiglitazone-treated group was pair-fed to match their daily food intake to that of the untreated fatty group. Lean Zucker rats ($n = 8$) were fed powdered diet, without rosiglitazone.

Blood pressure was measured in the conscious state by the tail-cuff method (IITC Model 179 Blood Pressure Analyzer; IITC/Life Science Instruments, Woodland Hills, CA) after prewarming the animals for 20 min at 30°C. To ensure reproducibility, two measurements were made, 10 and 7 days before killing the animals, and the mean value was used.

The rats were killed by cervical dislocation after a 12-h fast, and blood was obtained by cardiac puncture for subsequent assay of plasma insulin, using a radioimmunoassay kit with rat insulin standards (Amersham International, Amersham, U.K.), and plasma glucose by an autoanalyzer (BM/Hitachi 717; Boehringer Mannheim, Mannheim, Germany) using the hexokinase/glucose-6-phosphate dehydrogenase method. Plasma total cholesterol and triglyceride concentrations were measured by cholesterol oxidase and glycerol-3-phosphate oxidase assays, respectively (BM/Hitachi 717).

Reagents. Norepinephrine hydrochloride (NE) and acetylcholine chloride (ACh) (Sigma, Poole, U.K.) were made up as fresh base solutions in physiologic salt solution (PSS): composition (millimoles per liter): NaCl, 119; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.5; $MgSO_4 \cdot 7H_2O$, 1.17; $NaHCO_3$, 25; KH_2PO_4, 1.18; EDTA, 0.026; glucose, 5.5. Insulin (Humulin-S, 100 U/ml; Eli Lilly, Indianapolis, IN) was diluted to 0.01, 0.1, and 1 mU/ml using distilled water immediately before use. To achieve the required concentration, 25 µl of the appropriate insulin solution was added to the 5-ml myograph bath to yield working insulin concentrations of 50, 500, and 5,000 mU/l, respectively. These concentrations were chosen to represent physiologic (50 mU/l) and supraphysiologic (500 mU/l) concentrations; the very high concentration of 5,000 mU/l was used to determine whether the insensitivity to insulin's vasodilator action previously observed in fatty Zucker rats (13) extended into this pharmacologic range. The insulin used is a solution of human insulin in a preservative (glycerin and *m*-cresol). Arterial reactivity to NE was not affected by the insulin preservative alone, diluted in distilled water to a concentration equivalent to that at the highest working insulin concentration (results not shown), confirming that osmotic or other effects of adding insulin in this way were negligible.

Assessment of vascular function. The mesentery was removed and placed immediately in PSS gassed with 5% CO_2 in O_2. Eight third-order mesenteric arteries from each animal were dissected free of fat and connective tissue, and each was mounted on two 40-µm diameter stainless-steel wires in an automated myograph (Cambustion, Cambridge, U.K.). This is based on the principle of the Mulvany myograph (31) and measures the tension generated in response to various stimuli. The vessels were incubated at 37°C in PSS and aerated continuously with 5% CO_2 in O_2. After a 1-h rest, the length-tension characteristics for each vessel were determined using the law of Laplace ($P = T/r$; where P is the transmural pressure, T is the tension, and r is the vessel radius). Each vessel was then set to the normalized diameter, i.e., that it would achieve at rest in vivo under a transmural pressure of 100 mmHg. This has been shown to be the diameter at which the greatest force is generated (32). The computer calculated the target tension that each vessel should develop in response to a maximal stimulus.

After equilibration, each vessel was contracted twice with 2-min exposure to 125 mmol/l potassium (KPSS; KCl replacing NaCl in PSS), and then maximally with 10 µmol/l NE in KPSS; the bath was washed 3 times with PSS between contractions. Any vessel failing to reach its precalculated target tension was rejected.

Assessment of ACh-induced relaxation. Endothelium-dependent relaxation was measured after equilibration and initial priming with KCl and NE. When a plateau tension had been achieved after submaximal preconstriction with NE (6 µmol/l), the vessels were relaxed by exposure to stepwise increases in ACh concentration (0.6–100 µmol/l). The bath was then washed out three times with PSS.

Insulin-induced relaxation. After verifying preserved endothelial function, each vessel was rested for 30 min and then exposed to stepwise increases in NE concentration (0.2–30 µmol/l) to determine the dose-response curve. The bath was subsequently washed three times with PSS, and the vessels were then exposed to PSS containing insulin at concentrations of 50, 500, or 5,000 mU/l; each concentration was studied in 16 vessels (2 per rat and then averaged for each rat) from each experimental group (i.e., untreated and treated fatty rats and untreated lean control rats). The NE dose-response curve was then repeated with insulin still present in the bath. To ensure that repeated studies did not impair contractility, 48 arteries (16 from each group) underwent two NE dose-response curves, 1 h apart, in the absence of insulin; these showed no significant time-related differences (data not shown).

Data interpretation and statistical analyses. Vasoconstriction in response to NE was expressed as a percentage of the maximal tension generated in response to 30 µmol/l NE. Relaxation in response to each concentration of ACh was expressed as the percentage reduction from the maximal tension generated in response to NE (6 µmol/l) alone. Relaxation in response to insulin was calculated as the percentage reduction in tension generated at a given NE concentration in the presence of insulin, compared with the tension in the absence of insulin.

Dose-response data were analyzed by Scheffé's multiple analysis (two-way analysis of variance [ANOVA]) using Arcus Pro-II (Dr. Iain Buchan, Medical Computing, Aughton, West Lancs, U.K.). Differences in body weights, metabolic parameters, and blood pressure between obese and lean groups were analyzed

TABLE 1
Physical characteristics and metabolic data

Group	Body weight (g)	Systolic blood pressure (mmHg)	Plasma insulin (ng/ml)	Triglycerides (mmol/l)	Glucose (mmol/l)	Glucose-to-insulin ratio (mmol/µg)	Total cholesterol (mmol/l)
Untreated fatty rats	550 ± 19*	147 ± 5*	28.7 ± 6*	6.8 ± 0.8	10.8 ± 1.9*	0.4	4.9 ± 0.4*
Treated fatty rats	602 ± 20*	123 ± 1†	7.0 ± 1.5†*	3.6 ± 0.3†*	11.3 ± 1.0*	1.6	3.8 ± 0.1‡§
Untreated lean rats	352 ± 15†	125 ± 2†	1.5 ± 0.4†	0.7 ± 0.1†	4.8 ± 0.9§	3.2	1.9 ± 0.1†

Data are means ± SE. *$P < 0.001$, ‡$P < 0.05$ vs. lean; †$P < 0.001$, §$P < 0.05$ vs. untreated fatty.

by one-way ANOVA and Tukey's honestly significant difference test for multiple comparisons using the Statistica software package. Data are given throughout as means ± SE.

RESULTS

Metabolic data and physical characteristics. These data are shown in Table 1. At the end of the study, treated fatty rats were slightly heavier than untreated fatty rats, but this difference was not significant ($P = 0.1$).

Systolic blood pressure was significantly higher in the untreated fatty group than in both other groups ($P < 0.001$ vs. lean; $P < 0.001$ vs. treated fatty). Treatment with rosiglitazone significantly lowered blood pressure ($P < 0.001$) in fatty rats to levels that did not differ significantly from those in lean rats ($P = 0.3$).

Fasting plasma insulin and triglyceride concentrations were significantly higher in the untreated fatty rats than in the lean control rats (both $P < 0.001$). Treatment of fatty rats with rosiglitazone significantly lowered insulin by 76% ($P < 0.01$), triglycerides by 53% ($P < 0.01$), and cholesterol by 22% ($P < 0.05$), although all these levels remained significantly higher than in lean rats (triglycerides, $P < 0.001$; insulin, $P < 0.01$; cholesterol, $P < 0.001$). Fasting glucose was twice as high in the untreated fatty rats with hyperinsulinemia, consistent with severe insulin resistance. Therapy effected no significant change in glucose, but a significant reduction in insulin, indicating enhanced insulin sensitivity.

Arterial diameters did not differ significantly between any of the groups (all $P > 0.3$): lean, 207.9 ± 12.1 μm; untreated fatty, 243.4 ± 13.2 μm; treated fatty, 221.6 ± 6.2 μm.

Vascular function

ACh-induced relaxation. Vessels from lean control rats showed progressive relaxation to ACh in arteries preconstricted with 6 μmol/l NE and achieved a maximum 74.3 ± 3.5% relaxation with ACh concentrations of 100 μmol/l (Fig. 1). In arteries from the control fatty rats, ACh-induced relaxation was significantly impaired across the whole range of ACh concentrations tested, reaching a maximum of 62.4 ± 3.4% relaxation (Fig. 1; $P < 0.01$ vs. lean). Rosiglitazone-treated rats displayed slightly but significantly greater maximal relaxation (66.5 ± 3.0%; Fig. 1) compared with the untreated fatty rats ($P = 0.01$); however, this was still blunted compared with the lean group ($P = 0.01$).

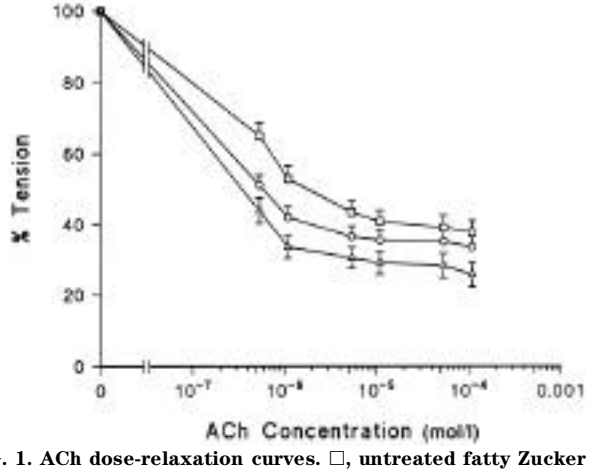

FIG. 1. ACh dose-relaxation curves. □, untreated fatty Zucker rats; ○, rosiglitazone-treated fatty Zucker rats ($P = 0.01$ vs. untreated fatty); △ untreated lean Zucker rats ($P = 0.01$ vs. rosiglitazone-treated fatty).

Vasoactive effects of insulin. Insulin at the lowest concentration (50 mU/l) significantly attenuated the vasoconstrictor response to NE in vessels from lean rats (14.7 ± 3.3% reduction in maximal tension in presence of insulin, $P = 0.02$; Fig. 2*A*). This effect of insulin was more pronounced at a concentration of 500 mU/l, with a maximum attenuation of 17.3 ± 2.2% ($P = 0.02$ vs. no insulin), but no greater at 5,000 mU/l (14.0 ± 1.7%; $P = 0.02$ vs. no insulin; data not shown).

The antispasmogenic effect of insulin was absent in the vessels from untreated fatty rats at all insulin concentrations studied (e.g., 50 mU/l; Fig. 2*B*).

Vessels from rosiglitazone-treated fatty rats showed partial restoration of the vasorelaxant effect of 50 mU/l insulin (9.7 ± 2.5% attenuation; $P = 0.02$ vs. no insulin; Fig. 2*C*). However, vessels from rosiglitazone-treated rats remained refractory to the antispasmogenic action of 500 mU/l and 5,000 mU/l insulin.

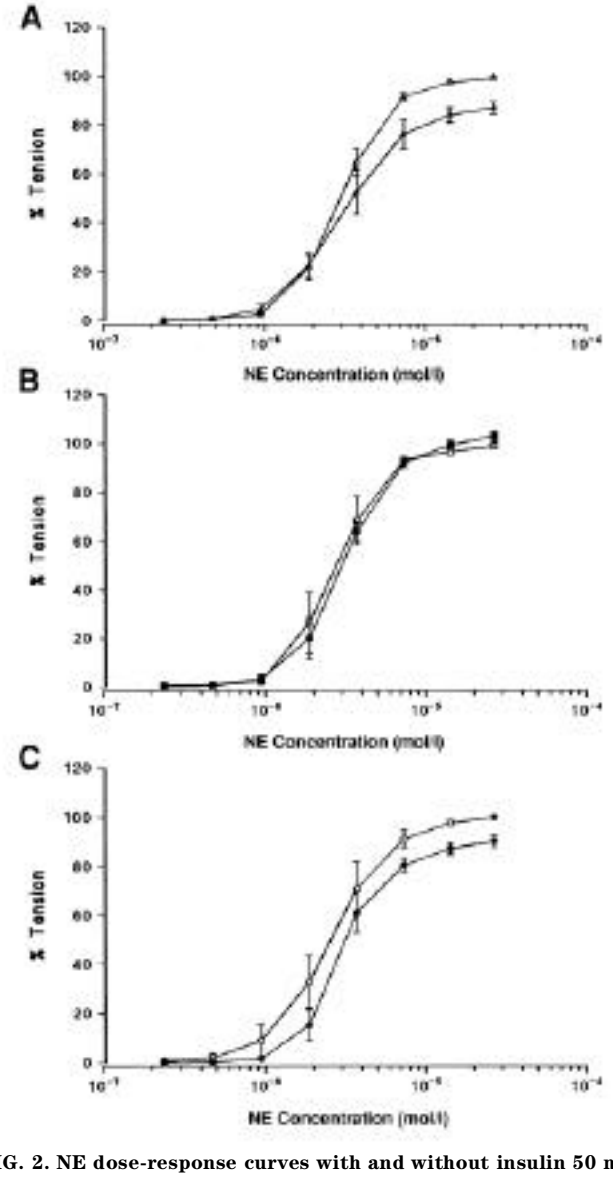

FIG. 2. NE dose-response curves with and without insulin 50 mU/l. *A*: Untreated lean Zucker rats: △, without insulin; ▲, with insulin 50 mU/l; $P = 0.02$ between the curves. *B*: Untreated fatty rats: □, without insulin; ■, with insulin 50 mU/l; NS between the curves. *C*: Rosiglitazone-treated fatty rats: ○, without insulin; ●, with insulin 50 mU/l; $P = 0.02$ between the curves.

The concentration-relaxation curves for all doses studied in the three groups of animals are shown in Fig. 3.

DISCUSSION

We have studied the possible role of insulin's vascular effects in the antihypertensive effect of the thiazolidinediones. Insulin has been shown to cause vasodilation in a number of vascular beds and causes hyperemia close to the site of subcutaneous injection (33). Injection of therapeutic doses of insulin may result in hypotension in patients with diabetic autonomic neuropathy (34). Dilatation of skeletal muscle arteries, capillary recruitment, and a significant increase in limb blood flow have all been demonstrated during hyperinsulinemic-euglycemic clamp studies in insulin-sensitive individuals (6), sometimes associated with a small but significant fall in blood pressure (3,6). Insulin infusion into phenylephrine-preconstricted dorsal hand veins causes local venous vasodilatation (35). Tooke et al. (36) have demonstrated dilatation of nail fold capillaries during insulin infusion in patients with type 1 diabetes. However, obesity, essential hypertension, and type 2 diabetes, states in which there is resistance to insulin-stimulated glucose uptake, are also associated with resistance to this vasodilator response to insulin in skeletal muscle (11,12) and in dorsal hand veins (35). At pharmacologic concentrations, insulin has been shown to cause vasodilatation in rodent mesenteric resistance arterioles (37). We have previously demonstrated that metabolic insulin resistance in the fatty Zucker rat is associated with impaired endothelium-dependent relaxation and loss of the ability of insulin to attenuate the vasoconstrictor action of NE in vitro (13).

Insulin levels raised as a result of metabolic insulin resistance might stimulate sympathetic activity (3,10,38) and, in the longer term, induce proliferation of vascular smooth muscle cells (39,40) and cause sodium retention (41), tending to raise blood pressure. Insulin's effects on sodium reabsorption and sympathetic activation are apparently preserved in insulin-resistant states (38,41).

Various thiazolidinediones have been shown to have antihypertensive properties. Troglitazone lowers systolic blood pressure in fatty Zucker rats (24), while pioglitazone attenuates diet-induced hypertension in Sprague-Dawley rats (27). Rosiglitazone prevented the age-related increase in blood pressure seen in the fatty Zucker rat (29).

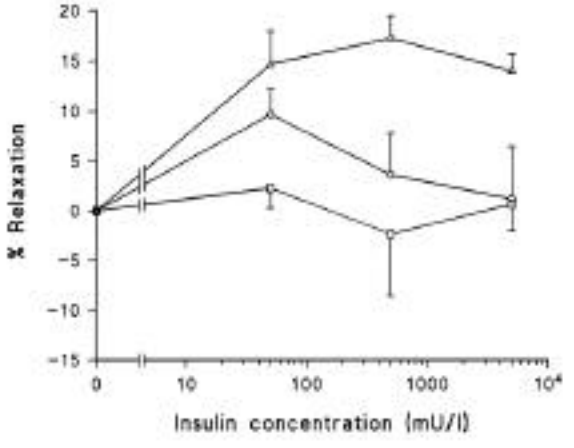

FIG. 3. Insulin concentration-relaxation curves in untreated lean (△), untreated fatty (□), and rosiglitazone-treated fatty (○) Zucker rats.

A number of studies in humans have demonstrated a blood pressure–lowering effect of troglitazone. Tack et al. (42) demonstrated a reduction in mean ambulatory 24-h diastolic blood pressure, but no effect on systolic blood pressure. Troglitazone resulted in a significant reduction in both systolic and diastolic blood pressure, as assessed by 24-h ambulatory monitoring, in normotensive insulin-resistant human subjects, accompanied by improved glucose tolerance and enhanced insulin sensitivity (20). In diabetic patients with essential hypertension, a significant reduction in sitting systolic and diastolic blood pressures was demonstrated, and this was associated with a lowering of plasma insulin and fasting glucose concentrations (43). In a 48-week study, troglitazone produced a significant lowering of systolic and diastolic blood pressures and a reduction in peripheral vascular resistance, calculated from the ratio of the mean arterial pressure to the cardiac output (44).

This study confirmed our previous findings that insulin at high physiological concentrations (50 mU/l) attenuates the vasoconstriction in response to NE in lean Zucker rats and that this effect of insulin is absent in fatty Zucker rats, even at the pharmacologic concentration of 5,000 mU/l (13). We have also confirmed that rosiglitazone (daily dose ~7 µmol/kg) improved insulin sensitivity in fatty Zucker rats, as shown by marked lowering of the fasting hyperinsulinemia. The moderate fasting hyperglycemia was not lowered, but the glucose-to-insulin ratio, an index of insulin sensitivity, rose fourfold with rosiglitazone. Moreover, we confirmed that treatment with rosiglitazone prevents the expected age-related rise in systolic blood pressure (15).

Our novel finding is that the impaired ACh-elicited endothelium-dependent vasorelaxation and decreased ability of insulin to attenuate NE-induced vasoconstriction are both partly corrected by rosiglitazone. The mechanism of this action is unknown at present, but we speculate that it may involve the drug's ability to lower cholesterol (25) and free fatty acid concentrations (18); oxidized LDL and free fatty acids have been shown to impair endothelium-dependent relaxation in rat aorta (45) and humans (46). We speculate also that insulin has a tonic vasodilator action in lean rats and that loss of this action in fatty Zucker rats may predispose to hypertension. From a previous study, it is clear that impairment of endothelial function precedes the development of hypertension (13). Partial restoration of insulin vasodilation by rosiglitazone in these animals constitutes a potential mechanism for preventing the rise in blood pressure seen in fatty control rats. The failure to demonstrate any effect of insulin at supraphysiologic concentrations (500 and 5,000 mU/l) in the rosiglitazone-treated group cannot be explained from the present data, but we argue that return of responsiveness to physiologic concentrations (i.e., 50 mU/l) is much more likely to be relevant. Interestingly, troglitazone has recently been demonstrated to improve endothelium-dependent relaxation in aortic segments from fructose-fed insulin-resistant Sprague-Dawley rats (47). Troglitazone failed to improve insulin-induced vasodilation in obese insulin-resistant subjects, in spite of an observed enhancement of insulin sensitivity (42). These apparently conflicting observations may be accounted for by interspecies variation or by differences in the methods used, in particular the caliber, and therefore the physiologic role of the vascular bed studied.

Other properties of thiazolidinediones may contribute to their blood pressure-lowering action. These include their inhibitory effects on vascular smooth muscle cell proliferation observed in vitro (48,49) and in vivo (26,49), which could counter the pathological changes in vessel function seen with insulin resistance and delay the onset of hypertension and vascular disease. In addition, troglitazone has been shown to increase skin blood flow in normal and dexamethasone-induced diabetic fatty Zucker rats, perhaps through increased generation of prostacyclin by the vascular endothelium (50). Pioglitazone attenuates the contractile responses to NE, arginine vasopressin, and potassium chloride in rat aortic rings in vitro (27), while both troglitazone and pioglitazone inhibit Ca^{2+} entry via L-type calcium channels in vascular smooth muscle cells (51,52), an action expected to result in vasodilatation. At present, there are no published data on the effects of rosiglitazone on vascular tone in animals either in vivo or in vitro, but, unlike troglitazone, it does not relax NE-preconstricted small arteries from human subcutaneous fat in vitro (53). At this stage, however, we cannot exclude the possibility of a direct vasodilator action of rosiglitazone in other, as yet unstudied, vascular beds. Finally, the antihypertensive effect of rosiglitazone in fatty Zucker rats may be related to its renal protective effect (25).

In conclusion, we postulate that the ability of rosiglitazone to prevent the development of hypertension in the fatty Zucker rat is related partly to the restoration of the tonic vasorelaxant action of insulin in resistance arteries, which would counterbalance the hormone's hypertensive effects. Rosiglitazone may help to reduce the risks of hypertension and ultimately of the cardiovascular disease associated with obesity and insulin resistance.

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