Altered Renal Microvascular Response in Zucker Obese Rats

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Although available evidence demonstrates that obesity manifests insulin resistance and causes glomerular sclerosis, it has not been determined whether insulin resistance alters the renal microvascular reactivity. This study examined whether insulin- and acetylcholine (ACH)-induced vasodilation was impaired in Zucker obese rats, and attempted to clarify the change in myogenic afferent arteriolar constriction, a determinant of glomerular pressure. Isolated perfused hydronephrotic rat kidneys were used to visualize the renal microcirculation. In Zucker lean rats, insulin (10 to 300 μU/mL) inhibited norepinephrine (NE)-induced afferent and efferent arteriolar constriction in a dose-dependent manner, with 112 % ± 8% and 98% ± 8% reversal at 300 μU/mL Similarly, ACH elicited dose-dependent dilation of these vessels. In Zucker obese rats, by contrast, afferent and efferent arterioles failed to dilate in response to insulin, and manifested diminished vasodilator responses to acetylcholine In the presence of nitro-L-arginine methylester (LNAME; 100 μmol/L), ACH (10 μmol/L) induced transient afferent arteriolar dilation (121% ± 9% reversal) in Zucker lean rats, whereas this response was blunted in obese rats (72% ± 8% reversal) Furthermore, myogenic afferent arteriolar constriction by elevating renal arterial pressure to 180 mm Hg was diminished in Zucker obese rats (-14% ± 3% decrement in diameter), compared with that in lean rats (-23% ± 2% decrement) Finally, the impairment in these vasodilator and vasoconstrictor responses was partially prevented by troglitazone, an insulin-sensitizing agent. Collectively, in insulin resistance, renal microvessels are refractory to the vasodilator action of insulin. Furthermore, "renal insulin resistance" is associated with the impaired vasodilator responses to ACH-induced nitric oxide (NO) and the diminished vasoconstrictor responses to pressure. The blunted myogenic afferent arteriolar constriction would allow glomerular hypertension, and in concert with the impaired endothelium-dependent vasodilation, could be responsible for the development of glomerular injury in obesity.

A GROWING BODY of evidence indicates that obesity is an important risk factor for the development of cardiovascular disorders. Recently, it has been suggested that insulin resistance constitutes an important determinant of the obesity-associated cardiovascular events.[1,2] Furthermore, insulin resistance not only elicits metabolic derangement in a variety of vascular beds, but also causes vasomotor alterations in systemic vascular beds. Thus, numerous studies have demonstrated that in insulin-resistant conditions, endothelium-dependent vasodilator responses are impaired in a variety of vasculature beds, including forearm[3] and coronary arteries.[4] It has also been demonstrated that insulin per se causes vasodilation mediated in part by nitric oxide (NO), [5,6] and that this action is diminished in systemic vascular beds of obese patients.[7] In the kidney, we have recently demonstrated that Zucker obese rats, in which insulin resistance develops, exhibit the impaired pressure-natriuresis response and systemic hypertension.[8] In this model of rats, renal interstitial NO production is greatly diminished. Although streptozotocin-induced insulin-deficient rats manifest impaired glomerular hemodynamics[9] as well as reduced glomerular NO production,[10] no investigations have evaluated the role of insulin resistance in mediating the altered glomerular hemodynamics.

Glomerular capillary pressure constitutes an important determinant of the development of renal injury.[11] Several lines of studies have established the role of afferent arteriolar tone in the regulation of glomerular capillary pressure.[12] The decreased afferent arteriolar resistance would facilitate the transmission of systemic blood pressure to the glomerulus. Thus, in diabetic nephropathy, afferent arteriolar resistance is reported to be reduced, leading to glomerular hypertension.[9] Similarly, in obese subjects, in which insulin resistance develops, glomerular filtration rate is reported to be elevated,[13] with glomerular sclerosis ensuing, probably as a result of glomerular hypertension. Furthermore, renal autoregulation is impaired in obesity,[8] suggesting blunted responsiveness to pressure of preglomerular arterioles. Nevertheless, no direct observations on myogenic (ie, pressure-induced) responsiveness of these vessels have been conducted in insulin resistance. Furthermore, whether amelioration of insulin resistance corrects the glomerular hemodynamics has not been examined.

In the present study, we examined whether the renal microvasculature was sensitive to the vasodilator action of insulin in Zucker obese rats, a model of obesity and insulin resistance, with the use of the isolated perfused hydronephrotic rat kidney.[6,14,15] Furthermore, the integrity of the renal microvascular endothelium was assessed by evaluating the acetylcholine (ACH)-induced vasodilation. Finally, the myogenic responsiveness of the afferent arteriole was evaluated to clarify whether insulin resistance was associated with altered glomerular hemodynamics in this rat strain.

METHODS

Isolated Perfused Hydronephrotic Kidney Studies

Chronic hydronephrosis was established to facilitate subsequent visualization of the renal microcirculation.[6,14,15] Six-week-old male Zucker obese and lean rats (Charles River Japan, Tokyo, Japan) were anesthetized with ether. The right ureter was ligated through a mid-abdominal incision. Then, the rats of each strain were allocated to 2

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groups according to the chow given; the rats were fed a standard (CE-10, Nippon Clea, Tokyo, Japan) or a troglitazone (30 mg/d)-containing diet, and were given water ad libitum. Following 8 to 10 weeks, at which time renal tubular atrophy had progressed to a stage that allowed direct microscopic visualization of renal microvessels,[16] the kidneys were harvested for perfusion study. Blood was drawn for measurement of serum glucose and insulin concentrations. All procedures were performed following the guidelines of the Animal Care Committee of Keio University.

On the day of the renal perfusion study, systolic blood pressure (SBP) was measured by the tail-cuff method (KN-210, Natsume, Tokyo, Japan). Thereafter, donor animals were anesthetized with ether, and the abdominal cavity was exposed by midline incision. The renal artery of the hydronephrotic kidney was cannulated in situ across the aorta through the superior mesenteric artery. Warm oxygenated medium was perfused throughout the cannulation procedure. The hydronephrotic kidney was excised and placed on the stage of an inverted microscope (IMT-2, Olympus, Tokyo, Japan) modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom surface. Kidneys were allowed to equilibrate for at least 30 minutes before initiating experiments.

Kidneys were perfused with media consisting of a Krebs-Ringer bicarbonate buffer containing 5 mmol/L D-glucose, 75% bovine serum albumin (Sigma, St Louis, MO), and a complement of amino acids.[17] The perfusion apparatus was illustrated in our previous publication.[14] The perfusion media (100 mL) was saturated with a gas mixture of $95\%O_2/5\%CO_2$ within a pressurized reservoir. The perfusion pressure, monitored at the level of the renal artery, was altered by adjusting the back-pressure–type regulator (Model 10BP, Fairchild Industrial Products, Winston-Salem, NC), which controlled the exit of gas from the media reservoir.

Vessel diameters were measured as detailed previously.[6,14,15] In brief, video images from a video camera (model XC-77, Sony, Tokyo, Japan) were recorded with a videocassette recorder and transmitted to a computer (PS55-5551, IBM Japan, Tokyo, Japan) equipped with a video display board (Targa 16+, Truevision, Indianapolis, IN). Vessel diameters were estimated with an automated program custom designed to permit determination of the mean distance between parallel edges of the selected vessels. A segment approximately 50 μm in length was scanned at 1- to 3-second intervals. Mean vessel diameter was determined by averaging all measurements obtained during the plateau of the response.

Experimental Protocols

Since afferent arteriolar constriction induced by norepinephrine (NE) is rather uniform, whereas that induced by pressure is strongest at the proximal portion of this vessel, the responses were evaluated at the midportion during NE-induced constriction, and near the interlobular artery during myogenic constriction Efferent arteriolar responses to ACH during NE-constriction were assessed near the glomerulus.

Insulin-induced arteriolar dilation in Zucker rats. The effects of insulin on NE-induced constriction of afferent and efferent arterioles were assessed Initially, NE (0.3 μmol/L) was added to the perfusion medium to obtain basal vascular tone. Following the determinations of baseline vasoconstrictor responses, increasing doses of insulin were administered (10, 30, 100, and 300 μU/mL), with an interval of 15 minutes between each dose of insulin. Finally, nitroprusside (10 μmol/L) was added to the perfusate to abolish the remaining vasoconstrictor tone induced by NE. To eliminate pressure-induced changes in vessel diameter, renal perfusion pressure was maintained constant at 80 mm Hg throughout the experimental protocol.

ACH-induced vasodilation in Zucker rats. The effect of ACH on NE-induced afferent and efferent arteriolar constriction was evaluated. Following the administration of 0.3 μmol/L NE, increasing doses of ACH (1 nmol/L to 10 μmol/L) were added directly into the perfusion medium, and the renal microvascular responses were assessed.

In the second series of experiments, the effect of ACH on NE-induced constriction was examined during the blockade of NO synthesis. Initially, nitro-L-arginine methylester (LNAME; 100 μmol/L) was added into the perfusion medium. Following the renal vasoconstriction by NE (0.3 μmol/L), renal arteriolar responses to ACH (1 nmol/L to 10 μmol/L) were assessed in both Zucker obese and lean rats.

Pressure- and NE-induced vasoconstrictions. The ability of afferent arterioles to constrict in response to elevated renal arterial pressure (RAP) was examined. Initially, RAP was maintained constant at 80 mm Hg. Then, RAP was raised in a stepwise manner by 20 mm Hg to 180 mm Hg to elicit myogenic tone of these vessels, and the arteriolar diameters were assessed at each RAP.

Next, afferent arteriolar responses to increasing doses of NE (0.03, 0.1, 0.3, and 1 μmol/L) were assessed in kidneys from both Zucker lean and obese rats.

Effect of troglitazone on arteriolar responses. Whether the pretreatment with troglitazone ameliorated the impaired insulin- and ACH-induced vasodilation and the blunted myogenic vasoconstriction was evaluated in kidneys from Zucker obese and lean rats. Following the induction of hydronephrosis, the rats were fed a diet containing troglitazone (30 mg/d) until the perfusion study (ie, for 8 to 10 weeks). On the day of the perfusion study, the renal microvascular responsiveness to insulin, ACH, and elevated renal perfusion pressure was examined in kidneys from these rats, and whether troglitazone ameliorated these responses was evaluated.

Isolated Perfused Normal Kidney Studies

The renal vasodilator action of insulin was examined with isolated perfused normal (ie,, nonhydronephrotic) kidneys under identical in vitro conditions.[14] Adult male Zucker obese and lean rats with or without 8 weeks of treatment with troglitazone (30 mg/d) were anesthetized with ether, and the renal artery was cannulated in situ. Upon cannulation, warm oxygenated medium was perfused, and the kidney was excised for perfusion study. Kidneys were allowed to equilibrate for at least 30 minutes before initiating experiments. Thereafter, the effect of insulin (10 to 300 μmol/L) on renal perfusate flow (RPF) under NE-constricted tone was evaluated with the use of electromagnetic flow probe (FF-015T, Nihon Kohden, Tokyo, Japan).

Analysis of Data

Results are expressed as the mean ± SEM. Data were analyzed by 1-way or 2-way analysis of variance (ANOVA), as appropriate, followed by Newman-Keuls post hoc test. P values less than 05 were considered statistically significant.

RESULTS

Isolated Perfused Hydronephrotic Kidney Studies

Systemic parameters. The body weight and SBP in Zucker obese rats (375 ± 15 g; 122 ± 3 mm Hg, n =24) were higher than those in Zucker lean rats (290 ± 9 g, $P < 05$; 111 ± 3 mm Hg, $P < .01$, n = 20; Table 1). The treatment with troglitazone tended to reduce SBP in Zucker obese rats, but had no effect on SBP in Zucker lean rats (Table 1).

Serum glucose levels in Zucker obese rats (9.5 ± 1.2 mmol/L, n = 24) tended to be greater than those in Zucker lean rats (6.9 ± 0.4 mmol/L, n = 20), but this difference did not attain statistical significance ($P > 05$) Zucker obese rats manifested elevated concentrations of serum insulin (181 ± 56 μU/mL) than that in Zucker lean rats (18 ± 5 μU/mL, $P <$ .01). These elevated levels were markedly decreased by trogli-

Table 1. Body Weight, Systolic Blood Pressure, and Laboratory Data in Zucker Rats

Rats	Body Weight (g)	SBP (mm Hg)	Serum Glucose (mmol/L)	Serum Insulin (μU/mL)
Lean (n = 20)	290 ± 9	111 ± 3	6.9 ± 0.4	18 ± 5
Lean + troglitazone (n = 10)	295 ± 6	102 ± 5	6.0 ± 0.6	10 ± 3
Obese (n = 24)	375 ± 15*	122 ± 3*	9.5 ± 1.2*	181 ± 56*
Obese + troglitazone (n = 13)	304 ± 14‡	114 ± 4	7.0 ± 0.9†	23 ± 7‡

NOTE. Values are means ± SEM. Lean indicates Zucker lean rats; and Obese indicates Zucker obese rats.
*$P < 0.01$ *v* lean.
†$P < .05$, ‡$P < 0.01$ *v* obese.

tazone treatment (7.0 ± 09 mmol/L and 23 ± 7 μU/mL, respectively) to those observed in Zucker lean rats.

Insulin-induced vasodilation. The vasodilator effect of insulin on NE-induced constriction of renal microvessels was assessed in both Zucker lean and obese rats In kidneys from Zucker lean rats, NE (0.3 μmol/L) elicited constriction of afferent (from 19.0 ± 0.6 to 12.4 ± 0.7 μm, $P < .01$, n = 7) and efferent arterioles (from 17.3 ± 0.5 to 11.8 ± 0.5 μm, $P < .01$, n = 7; Fig 1). In Zucker obese rats, renal arterioles exhibited marked constriction (afferent, from 190 ± 0.5 to 12.4 ± 0.5 μm, $P < .01$, n = 7; efferent, from 17.3 ± 0.5 to 12.3 ± 0.3 μm, $P < .01$, n = 7), similar in magnitude to that in Zucker lean rats ($P < .01$). When left untreated, these vasoconstrictor responses persisted throughout the experimental protocols (ie, 45 minutes.[6]

In Zucker lean rats, insulin was very potent in causing sustained inhibition of the NE-induced constriction of renal microvessels. Thus, 10 μU/mL insulin elicited dilation of both afferent (to 15.7 ± 0.6 μm, $P < .05$, n = 7) and efferent arterioles (to 14.4 ± 0.6 μm, $P < .05$, n = 7), which persisted for at least 20 minutes. Further addition of insulin reversed the NE-induced constriction in a dose-dependent manner. In contrast, insulin had no significant effect on NE-constricted afferent (n = 7) or efferent arterioles (n = 7) in Zucker obese rats. The subsequent addition of nitroprusside completely abolished the NE-induced constriction of afferent (to 19.5 ± 0.6 μm) and efferent arterioles (to 17.6 ± 0.6 μm).

The effect of insulin on RPF was compared in kidneys from Zucker obese and lean rats (Fig 1, bottom). In kidneys from Zucker lean rats, insulin potently reversed the NE-induced reduction in RPF, with 85% ± 9% reversal attained at 300 μU/mL. In contrast, RPF in Zucker obese kidneys was unaltered by insulin. The subsequent addition of nitroprusside completely restored the NE-induced reduction on RPF.

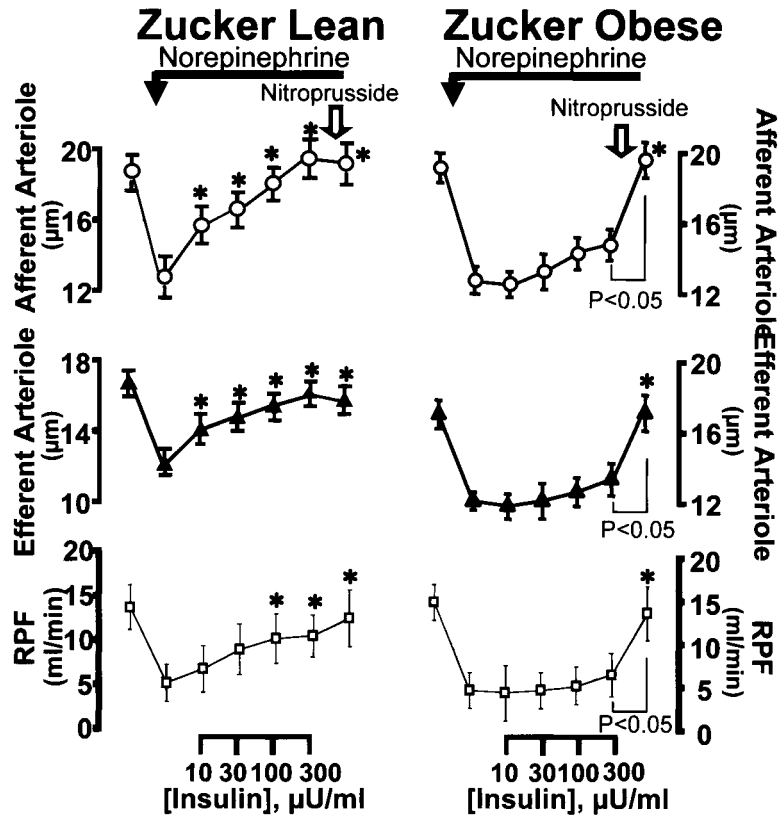

Fig 1. Effect of insulin on NE-induced constriction of renal microvessels in Zucker rats. NE (0.3 μmol/L)-induced decreases in afferent and efferent arteriolar diameters and RPF were reversed by insulin in a dose-dependent manner in Zucker lean rats, but these responses were blunted in Zucker obese rats. Values are means ± SEM. *$P < .05$ *v* NE.

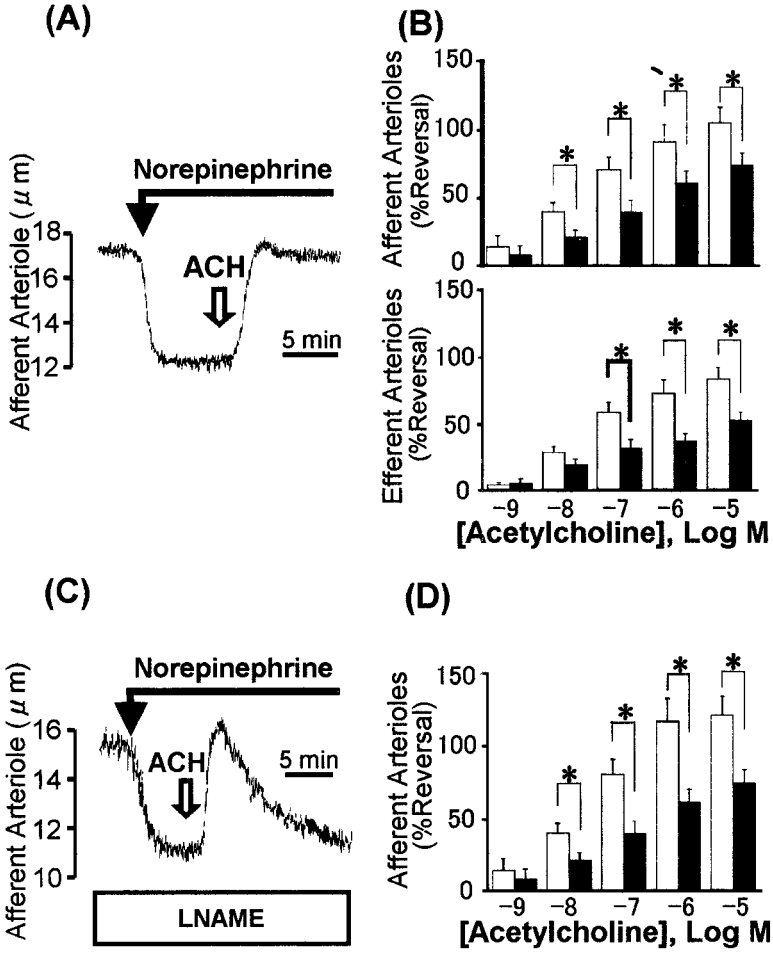

Fig 2. Effect of ACH on NE-induced constriction of renal microvessels. (A) Representative tracings showing a sustained vasodilator response of an afferent arteriole to ACH (10 μmol/L) in a Zucker lean rat. (B) Graphs summarizing the ACH-induced vasodilation of afferent and efferent arterioles in Zucker lean (□) and obese rats (■). (C) Representative tracings illustrating the ACH (10 μmol/L)-induced transient vasodilation of an afferent arteriole during LNAME treatment in a Zucker lean rat. (D) A graph comparing the transient afferent arteriolar vasodilation by ACH during LNAME treatment in Zucker lean (□) and obese rats (■). Values are means ± SEM. *$P < .05$.

ACH-induced vasodilation. The effect of ACH on NE-induced vasoconstriction of renal microvessels was evaluated. As depicted in Fig 2A, ACH (10 μmol/L) caused sustained dilation of an afferent arteriole in Zucker lean rats. In seven Zucker lean rats (Fig 2B, open bars), the ACH elicited vasodilation of both afferent and efferent arterioles in a dose-dependent manner, with 101% ± 8% and 84% ± 7% reversal observed at 10 μmol/L, respectively. In contrast, the responses to ACH in the obese littermate were diminished (Fig 2B, filled bars); at 10 μmol/L, ACH produced 72% ± 6% (n = 6) and 51% ± 5% reversal (n = 6) of NE-constricted afferent and efferent arterioles, respectively.

In the presence of LNAME, the sustained nature of the ACH-induced afferent arteriolar vasodilation was converted to transient dilation (Fig 2C). When the initial phase of the ACH-induced vasodilation was compared, afferent arterioles in Zucker obese rats (Fig 2D, filled bars; n = 6) exhibited less dilator responses to ACH than those in Zucker lean rats (open bars; n = 6). In contrast, the sustained phase of afferent arteriolar dilation was abolished to nearly the same levels in Zucker lean and obese rats; at 10 μmol/L, ACH produced only 22% ± 6% (n = 6) and 14% ± 6% reversal (n = 6) in Zucker lean and obese rats, respectively. In the efferent arterioles, LNAME (100 μmol/L) completely abolished the ACH (10 μmol/L)-induced vasodilation in both Zucker lean and obese rats.

Pressure- and norepinephrine-induced constriction of afferent arterioles. Although elevated RAP elicited myogenic vasoconstriction of the afferent arteriole in both Zucker lean and obese rats, the ability of this vessel to constrict was impaired in obese rats (Fig 3, left). Thus, afferent arterioles of Zucker lean rats manifested pressure-dependent constriction, with significant constriction observed at 100 mm Hg (from 19.0 ± 0.5 to 17.3 ± 0.6 μm, $P < .05$, n = 7). Further increments in RAP to 180 mm Hg elicited 23% ± 2% decrement in afferent arteriolar diameter (ie, to 14.7 ± 0.4 μm, $P < .01$). In contrast, the renal microvessels from Zucker obese rats exhibited markedly diminished response to pressure. Thus, 140 mm Hg of RAP was required to obtain the myogenic constriction of afferent arterioles (ie, from 18.7 ± 0.4 to 17.2 ± 0.3 μm, $P < .05$, n = 7), ie, 40 mm Hg higher than the RAP subtending the significant vasoconstriction in Zucker lean rats. At 180 mm Hg, afferent arterioles in Zucker obese rats constricted by only 14% ± 3% ($P < .05$ *v* Zucker lean rats).

As illustrated in Fig 3 (right), NE caused similar magnitude of afferent arteriolar constriction in Zucker lean and obese rats. Thus, 0.03 μmol/L NE elicited 13% ± 3% (from 18.1 ± 0.8 to 15.8 ± 0.6 μm, $P < .05$, n = 6) and 9% ± 3% (from 18.5 ±

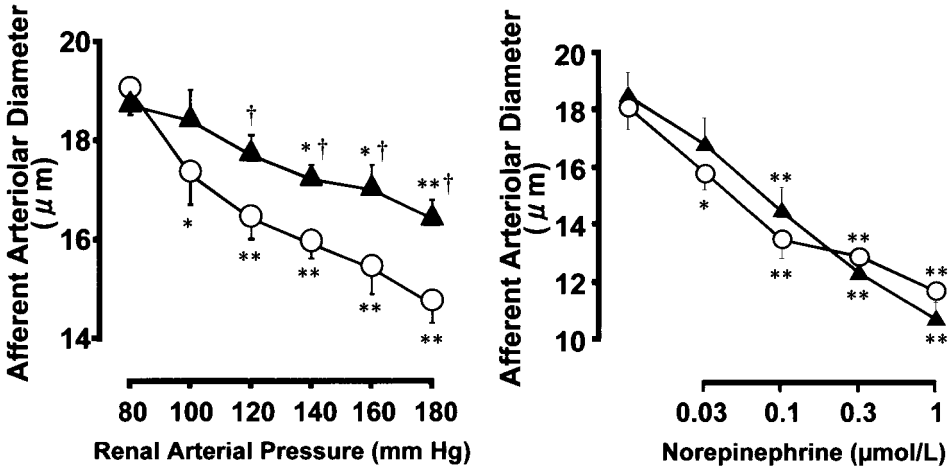

Fig 3. Effect of elevated RAP and NE on afferent arteriolar diameters. ○, Zucker lean rats ▲, Zucker obese rats. Values are means ± SEM. *p < .05; **P < .01 *v* 80 mm Hg (left) or baseline (right). †P < .05 *v* Zucker lean rats.

0.8 to 16.8 ± 0.9 μm, P = .07, n = 6) decreases in arteriolar diameter in Zucker lean and obese rats, respectively. Further addition of NE resulted in concentration-dependent constriction of this arteriole; at 1 μmol/L, NE caused 35% ± 6% (to 11.7 ± 0.8 μm, P < .05) and 42% ± 6% constrictor responses (to 10.7 ± 0.8 μm, P < .01) in Zucker lean and obese rats, respectively.

Effect of troglitazone on arteriolar responses. In Zucker lean rats (n = 5), the pretreatment with troglitazone had no effect on insulin (300 μU/mL)-induced increases in afferent and efferent arteriolar diameters (Fig 4) or on RPF (81% ± 9% *v* 79% ± 9% reversal in the absence of troglitazone). In Zucker obese rats (n = 6), however, troglitazone partially restored the insulin-induced vasodilation of these vessels (afferent, 73% ± 6% *v* 21% ± 3% reversal, P < .01; efferent, 75%.± 8% *v* 20% ± 3% reversal, P < .01) and the increase in RPF (65% ± 9% *v* 18% ± 7% reversal, P < .01). In analogy, troglitazone restored the impairment in both initial and sustained phases of afferent arteriolar dilation, as well as in efferent arteriolar responses to ACH in Zucker obese rats.

Troglitazone pretreatment did not alter the myogenic vasoconstriction of afferent arterioles in Zucker lean rats (n = 5; Fig 4). In contrast, in Zucker obese rats, troglitazone markedly improved the myogenic vasoconstrictor responses. Thus, elevating RAP to 180 mm Hg elicited 19% ± 4% vasoconstriction (from 19.3 ± 0.4 to 15.7 ± 0.3 μm, P < .01, n = 7), a value nearly identical to that observed in Zucker lean rats.

Isolated Perfused Normal Kidney Studies

The effect of insulin on RPF was evaluated in isolated perfused normal (nonhydronephrotic) kidneys from Zucker

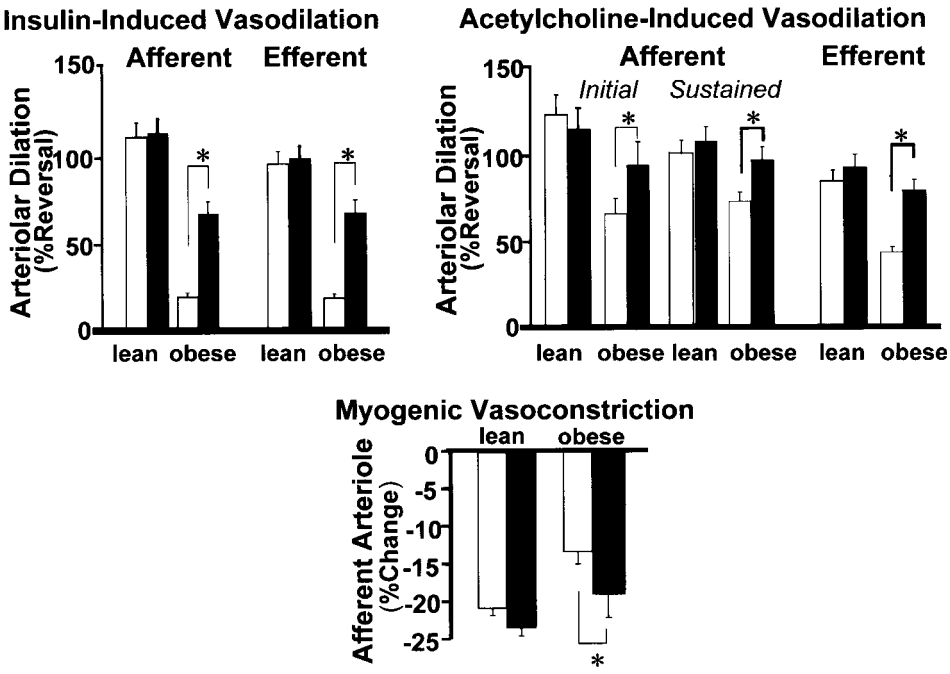

Fig 4. Effects of troglitazone on insulin- and ACH-induced vasodilation, and myogenic vasoconstriction of renal microvessels. □, Control rats; ■, troglitazone-treated rats. Values are means ± SEM. *P < .05.

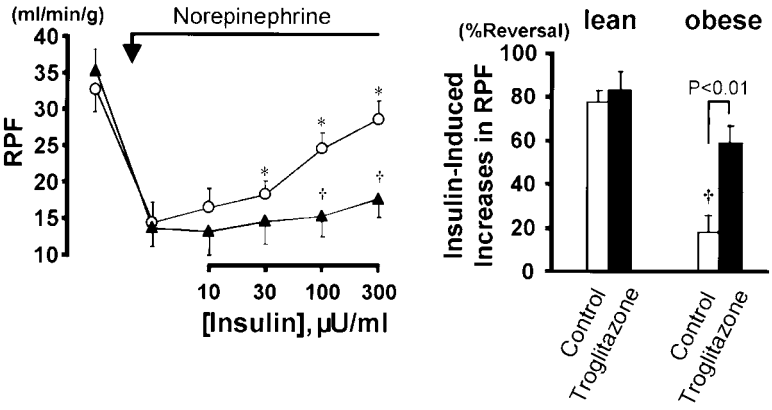

Fig 5. Changes in renal perfusate flow in Zucker obese rats. Insulin dose-dependently restored the NE-induced decreases in RPF in Zucker lean rats (○), but this response was markedly blunted in Zucker obese rats (▲, left). Eight weeks of treatment with troglitazone prominently prevented the impairment in insulin-induced changes in RPF in Zucker obese rats (right). Values are means ± SEM. *$P < .05$ *v* NE. †$P < .05$ *v* Zucker lean rats.

obese and lean rats (Fig 5). In Zucker lean rats, insulin restored the NE-induced decreases in RPF in a dose-dependent manner; 78% ± 5% reversal was observed at a dose of 300 μU/mL In contrast, RPF failed to increase in Zucker obese rats. This impaired response to insulin was prevented by 8 weeks of treatment with troglitazone (Fig 5, right).

DISCUSSION

It is well known that glomerular sclerosis develops in obesity. Several factors are assumed responsible for the development of glomerular sclerosis in obesity, including hypertension and deranged lipid metabolism.[18] Furthermore, it has recently been suggested that insulin resistance per se constitutes an important determinant of the obesity-associated hemodynamic impairment. Thus, insulin resistance is closely linked to systemic hypertension.[19,20] Indeed, in insulin resistance pressure-natriuretic response is impaired,[8] which would subsequently elicit hypertension. Furthermore, endothelium-dependent vasodilation is blunted in forearm[3] and coronary arteries in insulin resistance.[4] In parallel with the latter observations indicating dysregulation in systemic vascular tone, the elevated glomerular filtration rate constitutes an important component characterizing the renal hemodynamics in obesity.[13] Nevertheless, no investigations have been conducted evaluating the mechanism of the altered glomerular hemodynamics in insulin resistance.

The present study demonstrates that Zucker obese rats exhibit metabolic abnormality associated with insulin resistance. Thus, higher concentrations of insulin are required to prevent hyperglycemia (Table 1). In such a setting, this rat strain manifests an impaired insulin-induced dilation of renal microvessels, whereas in the lean littermate insulin prominently dilates these vessels (Fig 1.) Previous studies demonstrated that insulin elicited the vasodilation of several vascular beds, including forearm vessels[21] and leg arteries.[22] In the renal microcirculation, we recently demonstrate that insulin causes dose-dependent dilation of renal arterioles in kidneys from Sprague-Dawley rats,[6] similar in magnitude to that in Zucker lean rats. Collectively, insulin is not only traditionally regarded as a metabolic hormone contributing to the regulation of carbohydrate metabolism, but also serves to act as a potent vasodilator and modulate renal vascular tone. More importantly, the diminished vasodilator response to insulin in Zucker obese rats could indicate insulin resistance within the renal microcirculation. Of note, the present study compared the renal hemodynamic action of insulin in hydronephrotic and normal (ie, nonhydronephrotic) kidneys, and found that the effect of insulin on RPF was nearly identical under these experimental conditions (Figs 1 and 5). Furthermore, we previously demonstrated that atrial natriuretic peptide produced qualitatively similar renal hemodynamic action in hydronephrotic and normal kidneys.[14] Our observations in the hydronephrotic kidney model would thus reflect at least in part the renal microvascular action of insulin in normal kidneys. Nevertheless, an important caveat is that hydronephrosis disrupts tubular function and therefore is predicted to modify renal hemodynamics.

Several lines of investigations have demonstrated vascular dysfunction in insulin resistance Miller et al[4] reported that the ACH-induced vasodilation was diminished in coronary arteries from fructose-fed insulin resistant rats. Furthermore, Walker et al [23] found that insulin-mediated dilation of the mesenteric artery was diminished in Zucker obese rats. The latter observation therefore coincides with the present finding demonstrating an impaired insulin-induced vasodilation. In this regard, Cleland et al[24] reported functional coupling between insulin action and basal endothelial NO production in type 2 diabetic patients. Furthermore, insulin upregulates endothelial nitric oxide synthase (eNOS) gene expression in the endothelial cell[5] Thus, insulin resistance is responsible for the vasomotor dysregulation, and the impairment in NO production may participate in part in the deranged vascular tone, as well as histologic damage,[25] in insulin resistance Indeed, we have recently demonstrated that insulin-induced vasodilation of renal microvessels is mediated in large part by the dilator action of NO in the same experimental model.[6] Furthermore, the present study shows that nitroprusside completely inhibits the NE-induced constriction of afferent arterioles (Fig 1). Thus, the diminished insulin-induced renal vasodilation in Zucker obese rats should be attributed in large part to the impaired renal endothelium-dependent NO production, but not to the damaged arteriolar smooth muscles. Finally, the current study demonstrates an impaired ACH-induced vasodilation of renal arterioles in this rat strain (see below). It is reasonable therefore to speculate that endothelial dysfunction is involved at least in part in the deranged vascular tone in insulin resistant Zucker obese rats. The altered intrarenal NO production is also observed in renal

cortex and medulla in Zucker obese rats, whereby the pressure-natriuresis is impaired.[8]

Endothelium has recently been established as an important structure releasing a variety of vasoactive substances. We have recently demonstrated that ACH-induced afferent arteriolar dilation comprises several components, including NO and endothelium-derived hyperpolarizing factor (EDHF). [26,27] In the present study, we further examined whether the role of these vasodilator substances was altered in afferent arterioles from hydronephrotic Zucker obese rats. Thus, both initial and sustained phases of the ACH-induced dilation were impaired in afferent arterioles from Zucker obese rats (Fig 2). Based on our previous observations that the initial phase of ACH-induced afferent arteriolar dilation is mediated mainly by EDHF, while the sustained phase is attributed to NO,[26] the present findings would represent an impaired EDHF, as well as NO, activity in afferent arterioles from insulin resistant rats. In this regard, Katakam et al[28] recently demonstrate that cytochrome P450–mediated EDHF production is impaired in mesenteric arteries of fructose-fed insulin-resistant rats. In contrast, we have reported a selective impairment in the EDHF-mediated component of ACH-induced afferent arteriolar dilation in spontaneously hypertensive rats, with preserved NO-mediated vasodilation.[27] Although systemic hypertension is less severe in Zucker obese rats than in spontaneously hypertensive rats in which glomerular injury is relatively spared, insulin resistance may cause more extensive endothelial impairment, which might be associated with the difference in the severity of glomerular injury between these rats.

Although the impairment of insulin-induced dilation favors renal vasoconstriction, several lines of studies rather demonstrate renal hyperperfusion in insulin resistance conditions. Dengel et al[13] reported that renal blood flow and glomerular filtration rate increased in obese hypertensive patients. Furthermore, in the renal micropuncture study, Park et al[29] reported an increase in single-nephron plasma flow in Zucker obese rats Thus, unlike other vascular beds showing increased vascular resistance,[30] renal microcirculation manifests reduced vascular tone in insulin resistant conditions. These contrasting observations suggest that the attenuated renal vasodilator effect of insulin cannot explain the altered renal hemodynamics in insulin resistance, but other mechanisms should constitute an important determinant of insulin resistance–mediated renal vasodilation. In the present study, we demonstrate an impaired myogenic vasoconstriction of preglomerular (ie, afferent) arterioles in Zucker obese rats (Fig 3). Our current finding therefore could explain the elevated glomerular filtration rate in this rat strain,[29] and further support the impaired renal blood flow autoregulation[8] since the myogenic vasomotor tone of preglomerular vessels contributes importantly to the maintenance of renal autoregulation.[31] Similar impairment in myogenic afferent arteriolar tone has been reported in hydronephrotic kidneys from diabetic rats induced by streptozotocin[32] and galactose-feeding.[33] In contrast, at the efferent arteriole devoid of myogenic response,[34] the diminution of insulin-/ACH-mediated dilation may favor efferent arteriolar constriction and glomerular hypertension.

The mechanism whereby insulin resistance is associated with impaired myogenic vasoconstriction remains undetermined. It is demonstrated that in mesangial cells, diminished intracellular Ca^{2+} increases in response to angiotensin II are attributed to the absence of insulin.[35] The present study shows that afferent arterioles in Zucker obese rats manifest impairment in myogenic vasoconstriction whereas the NE-induced constriction is preserved. Of note, we have recently demonstrated that elevated RAP activates mechanosensitive cation channels, and subsequently opens voltage-dependent calcium channels within the afferent arteriole.[36] Furthermore, the inhibition of voltage-dependent calcium channels by calcium antagonists completely inhibits NE-induced vasoconstriction of this arteriole.[37] It therefore appears less likely that insulin resistance directly alters the function of voltage-dependent calcium channels per se, but factors modulating these calcium channels, including mechanosensitive cation channels and 20-hydroxyeicosatetraenoic acid, a modulator of myogenic response,[38] may be affected by insulin resistance These speculations however require further investigations.

The role of altered myogenic afferent arteriolar vasoconstriction in the development of renal injury merits comment. It has been suggested that the impaired myogenic tone is associated with deranged renal hemodynamics and subsequent glomerular injury in a variety of experimental models, including Dahl salt-sensitive rats,[39] and renovascular hypertension.[40] Similarly, the kidney from streptozotocin-induced diabetic rats also manifests impaired myogenic response of the afferent arteriole[32] and glomerular injury.[41] The latter observation is noteworthy since this rat manifests diminution of insulin action, and thus parallels our present finding that myogenic afferent arteriolar constriction is impaired in insulin-resistant Zucker obese rats. In concert, the derangement in carbohydrate metabolism causes afferent arteriolar dysfunction, which allows the transmission of the systemic blood pressure to the glomerulus, leading to glomerular hypertension. In this regard, we have recently demonstrated increased proteinuria in Wistar fatty rats before the development of hypertension.[42] Since these rats share the property of insulin resistance and obesity[42] with Zucker obese rats, the alteration in glomerular hemodynamics by insulin resistance, rather than hypertension per se, may be responsible for the development of proteinuria[43] and possibly renal arteriolar dysfunction.

The present study demonstrates that the correction of insulin resistance by troglitazone restores the vasodilator action of insulin in renal microvessels from Zucker obese rats (Fig 4). In the same experimental setting, troglitazone not only improves the ACH-induced vasodilation but also restores myogenic vasoconstrictor responsiveness. The latter finding is of particular interest, since the amelioration of the myogenic vasoconstriction would prevent the direct transmission of systemic blood pressure to the glomerulus. Indeed, we recently demonstrate that troglitazone restores the renal blood flow autoregulation in this rat strain.[8] It is therefore anticipated that troglitazone could reduce glomerular capillary pressure Of interest, whereas only modest SBP reductions by troglitazone in Zucker obese rats (122 ± 3 *v* 114 ± 4 mm Hg; $P > .05$; Table 1) may affect endothelial function, more marked decreases in serum insulin (181 ± 56 *v* 23 ± 7 μU/mL, $P < .01$) suggest that insulin

resistance within the renal microvasculature, rather than systemic hypertension, constitutes a determinant of renal arteriolar tone, and may thus modify the glomerular hemodynamics. Alternatively, peroxisome proliferator-activated protein γ (PPARγ) activation by troglitazone may directly improve the renal function.[44,45] We propose that the insulin-sensitizing agent possesses not only antidiabetic action, but also vasculoprotective effect under the circumstance with insulin resistance.

In conclusion, the present study demonstrates that insulin-induced vasodilation of renal microvessels is prominently blunted in hydronephrotic kidneys from Zucker obese rats, suggesting insulin resistance in the renal microvasculature. Furthermore, these microvessels manifest impaired responsiveness to alterations in renal perfusion pressure Such deranged myogenic responsiveness may be responsible for the impaired renal autoregulation and renal hyperfiltration, and would allow systemic blood pressure to the glomerulus, thus favoring glomerular hypertension. Finally, the correction of insulin resistance by troglitazone ameliorates the vascular insulin resistance and myogenic vasoconstriction. This therapeutic tool therefore may constitute an important strategy for the prevention of glomerular sclerosis in insulin resistance conditions.

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