Renal Protective Effect of Troglitazone in Wistar Fatty Rats

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Although it is known that renal injury develops in obesity and diabetes mellitus, there have been no investigations examining the impact of insulin resistance per se on the development of renal injury. The present study was undertaken to examine whether insulin resistance and obesity influence urinary protein excretion (UPE) in female heminephrectomized Wistar fatty rats (WFRs). After 24 weeks of heminephrectomy in WFRs, the body weight ([BW], 465 ± 18 g; n = 6), blood pressure (155 ± 5 mm Hg), serum insulin to glucose ratio (1.31 ± 0.39 μU/mg), and daily UPE (24 ± 7 mg/d) were greater versus Wistar lean rats ([WLRs] 258 ± 8 g, 134 ± 1 mm Hg, 0.19 ± 0.06 μU/mg, and 5 ± 1 mg/d, respectively; n = 6), whereas blood glucose levels did not increase significantly. In WFRs, long-term (ie, 24 weeks) treatment with troglitazone, an insulin-sensitizing agent, improved the serum insulin to glucose ratio (0.17 ± 0.09 μU/mg), reduced blood pressure (to 140 ± 4 mm Hg), and decreased UPE (to 7 ± 1 mg/d), although it had no effect on BW. Of note, with troglitazone treatment, the reduction in proteinuria preceded the correction of hypertension (ie, at week 12). In conclusion, our study suggests that insulin resistance per se causes proteinuria that does not appear to depend on blood pressure. Furthermore, long-term therapy with troglitazone may be a useful tool for the treatment of renal injury in the insulin-resistant condition.

A GROWING BODY OF EVIDENCE has indicated that obesity and insulin resistance are important risk factors for the development of a variety of cardiovascular disorders.[1,2] It has been recognized that obesity and insulin resistance not only manifest metabolic derangement but also cause hemodynamic alterations. Indeed, in the insulin-resistant condition, the endothelium-dependent vasodilator response is blunted.[3,4] Furthermore, insulin resistance impairs renal sodium excretory mechanisms.[5,6] Such alterations in hemodynamic regulatory mechanisms would elevate systemic blood pressure and may subsequently cause renal injury.[7,8] Although diabetes mellitus, a disorder of diminished insulin action, is well known as a metabolic disease manifesting alterations in systemic and renal hemodynamics and renal injury, little is known as to the role of insulin resistance per se in mediating the renal impairment.

Recently, many types of orally active antidiabetic agents have been developed. Troglitazone is a unique insulin-sensitizing agent that ameliorates hyperglycemia and corrects insulin resistance. Furthermore, several studies have revealed that troglitazone improves hypertension,[9,10] although the mechanism for the depressor action of this agent remains unclarified. In a recent study, we have demonstrated that in obese Zucker rats, the correction of insulin resistance by a 4-week treatment with troglitazone completely abolishes hypertension and partially improves the blunted pressure-natriuresis and the renal interstitial nitrite/nitrate (metabolite of nitric oxide) levels.[5] Furthermore, troglitazone has recently been reported to ameliorate microalbuminuria in diabetic nephropathy.[11] It is therefore reasonable to speculate that troglitazone may affect renal function. However, to our knowledge, no investigations have examined the long-term effect of troglitazone on renal injury in the nondiabetic, insulin-resistant condition.[12]

In the present study, we examined the role of insulin resistance and obesity in the development of renal injury. Furthermore, the long-term effect of troglitazone on proteinuria and hypertension was assessed. To clarify these issues, we studied female Wistar fatty rats (WFRs) with heminephrectomy. This animal model manifests insulin resistance and obesity but not overt diabetes.[13] Furthermore, heminephrectomy, which causes glomerular hyperfiltration,[14] would facilitate the impact of insulin resistance and obesity on the kidney.

MATERIALS AND METHODS

Animal Preparation

All experimental protocols were conducted according to the guidelines of the Animal Care Committee of Keio University. Seven-week-old female WFRs and their controls (Wistar lean rats [WLRs]) were used. The rats were divided into the following groups: (1) WLR (n = 6), (2) WLR + troglitazone (n = 6), (3) WFR (n = 6), and (4) WFR + troglitazone (n = 6). To facilitate the comparison of the effect of insulin resistance on blood pressure and urinary protein excretion (UPE) the right kidney was removed 5 days before initiation of the experimental protocols. All rats were placed in metabolic cages and were fed a standard rat chow (0.38% Na, 0.97% K, and 25.1% protein; Nippon Clea, Tokyo, Japan) with or without troglitazone (30 mg/d; Sankyo Pharmaceutical, Tokyo, Japan) for 24 weeks. Troglitazone was administered by adding it to the chow. The animals were allowed free access to tap water throughout the experimental protocols.

Metabolic Study

Body weight (BW), systolic blood pressure ([SBP] measured by the tail-cuff method, KN-210; Natsume, Tokyo, Japan), and 24-hour UPE were evaluated at 0, 12, 18, and 24 weeks.[15] At 24 weeks, all rats were decapitated and the blood was collected for measurement of serum glucose, insulin, total protein, urea nitrogen, and creatinine.

Statistics

Results are expressed as the mean ± SEM. Statistical analysis was performed by 2-way ANOVA with repeated measures, followed by a multiple-comparison post hoc test. A P value less than .05 was considered statistically significant.

RESULTS

Laboratory Data

Serum creatinine, total protein, and urea nitrogen did not differ among the groups (Table 1). The serum glucose concentra-

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Table 1. Laboratory Data

Group	Serum Glucose (mg/dL)	Serum Insulin (μU/mL)	Serum Insulin/Glucose Ratio (μU/mg)	Total Protein (mg/dL)	Serum Creatinine (mg/dL)	Blood Urea Nitrogen (mg/dL)
WLR (n = 6)	96 ± 5	19 ± 7	0.19 ± 0.06	5.8 ± 0.1	0.50 ± 0.04	22.4 ± 1.7
WLR + troglitazone (n = 6)	119 ± 7	6 ± 1	0.05 ± 0.01	6.2 ± 0.1	0.52 ± 0.04	26.9 ± 1.0
WFR (n = 6)	157 ± 26	222 ± 78*	1.31 ± 0.39*	5.7 ± 0.3	0.56 ± 0.16	32.9 ± 7.1
WFR + troglitazone (n = 6)	120 ± 12	20 ± 12†	0.17 ± 0.09†	5.6 ± 0.2	0.60 ± 0.08	39.5 ± 9.1

NOTE. Values are the mean ± SEM.
*$P < .05$ *v* WLR.
†$P < .05$ *v* WFR.

tion was higher in WFRs versus WLRs, although it did not attain statistical significance. WFRs manifested a markedly elevated serum insulin and insulin to glucose ratio, a marker of insulin resistance.[5] These elevated levels in WFRs were greatly decreased by the treatment with troglitazone.

BW

Figure 1 illustrates the changes in BW in WFRs and WLRs. BW was increased in WFRs (n = 6) in comparison to WLRs (n = 6) throughout the study. Troglitazone did not influence the BW changes in either WFRs (n = 6) or WLRs (n = 6).

SBP

SBP in WLRs did not change throughout the experiments (n = 6; Fig 2). In contrast, in WFRs (n = 6), SBP was markedly elevated, attaining a level of 162 ± 5 and 155 ± 5 mm Hg at week 18 ($P < .05$ *v* WLRs) and week 24 ($P < .05$ *v* WLRs), respectively.

Treatment with troglitazone had no effect on SBP in WLRs (ie, WLR + troglitazone, $P > .5$, n = 6). However, in WFRs, troglitazone markedly suppressed the increase in SBP (18 weeks, 139 ± 5 mm Hg, $P < .05$; 24 weeks, 140 ± 4 mm Hg, $P < .05$; n = 6). Thus, SBP in troglitazone-treated WFRs was maintained nearly constant at the level observed in WLRs ($P > .5$).

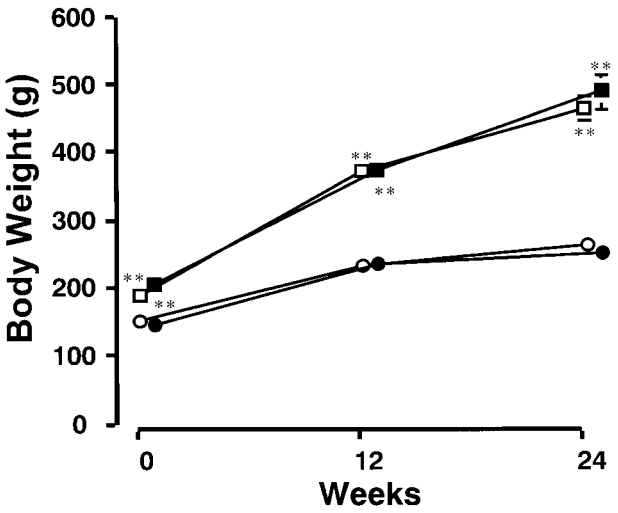

Fig 1. Changes in BW in WFRs and WLRs. BW was greater in WFRs with (■) and without troglitazone (□) *v* their WLR counterparts (with troglitazone, ●; without troglitazone, ○). Values are the mean ± SEM. **$P < .01$ *v* WLRs.

UPE

Figure 3 illustrates the effect of troglitazone on UPE in WLRs and WFRs. In WLRs with heminephrectomy, UPE did not increase throughout the protocol period ($P > .5$, n = 6). In contrast, UPE in WFRs significantly increased from 2 ± 1 mg/d at week 0 to 7 ± 2 mg/d at week 12 ($P < .05$, n = 6). Of note, SBP was not elevated at this stage. Further increases in UPE were observed at week 18 (12 ± 3 mg/d, $P < .01$, n = 6) and week 24 (24 ± 7 mg/d, $P < .01$, n = 6), values higher than those in WLRs (week 12, $P < .01$; week 18, $P = .06$; week 24, $P < .01$).

The treatment with troglitazone had no effect on protein excretion in WLRs (ie, WLR + troglitazone, $P > .5$, n = 6). In contrast, troglitazone markedly improved proteinuria in WFRs (ie, WFR + troglitazone). Thus, troglitazone suppressed the increase in proteinuria at week 12 (2 ± 1 mg/d, $P < .01$, n = 6), week 18 (7 ± 3 mg/d, $P = .09$, n = 6), and week 24 (7 ± 1 mg/d, $P < .05$ *v* WFR).

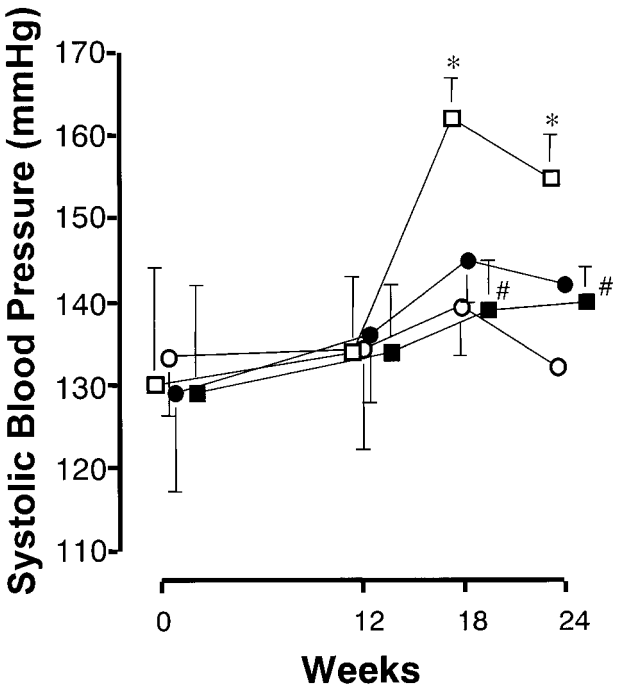

Fig 2. Alterations of SBP in WFRs and WLRs. SBP was significantly greater in WFRs without troglitazone (□) *v* WLRs without troglitazone (○) at 18 and 24 weeks. Troglitazone markedly reduced SBP in WFRs (■), whereas it had no effect on SBP in WLRs (●). Values are the mean ± SEM. *$P < .05$ *v* WLRs, #$P < .05$ *v* WFRs.

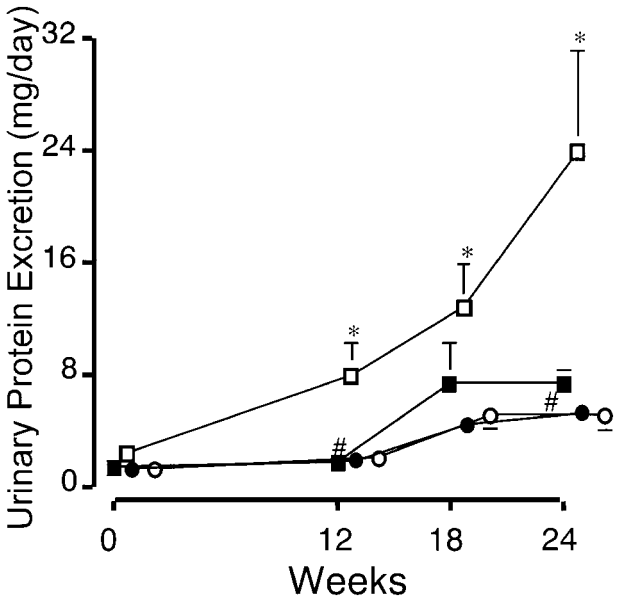

Fig 3. Changes in UPE in WFRs and WLRs. UPE in WFRs significantly increased at 12, 18, and 24 weeks (□). These elevations in UPE were suppressed by treatment with troglitazone (■). ○, WLRs; ●, WLRs with troglitazone. Values are the mean ± SEM. *$P < .05$ *v* WLRs, #$P < .05$ *v* WFRs.

DISCUSSION

Several lines of clinical investigation have facilitated the recognition of insulin resistance as a cause of various cardiovascular diseases.[1,2] Furthermore, insulin resistance is reported to be closely associated with hypertension in several experimental models, including Zucker obese rats[5,7] and WFRs.[6,13] In these rats, renal injury develops,[8,16,17] ostensibly as a result of systemic hypertension. However, no investigations have determined the role of insulin sensitivity in the progression of renal injury. Furthermore, although an insulin-sensitizing agent is reported to ameliorate microalbuminuria in diabetes mellitus,[18] it remains undetermined whether this class of the agent possesses renal protective action in the nondiabetic insulin-resistant condition.

In the present study, we have demonstrated that the female WFR, characterized by obesity and insulin resistance, manifests hypertension and proteinuria when the animal is heminephrectomized. In contrast, proteinuria does not develop in the WLR with heminephrectomy. Furthermore, treatment with troglitazone markedly improved these changes. It has been documented that obesity and insulin resistance are important risk factors for the development of hypertension.[19] A growing body of evidence shows that the kidney plays a pivotal role in the development of hypertension in the insulin-resistant condition.[19,20] We have recently demonstrated the impaired pressure-natriuresis response and hypertension in Zucker obese rats[5] and WFRs,[6] both of which represent an animal model of insulin resistance. Furthermore, the improvement of insulin resistance by troglitazone leads to the partial amelioration of the pressure-natriuresis response and correction of hypertension in Zucker obese rats.[5] The present observation demonstrating the association between insulin resistance and proteinuria suggests an important role of insulin sensitivity in mediating the development of renal injury. Furthermore, the observation that proteinuria precedes the development of hypertension (ie, at week 12) favors a more important role of insulin resistance per se, rather than hypertension, in causing proteinuria.

The mechanisms for the development of proteinuria in insulin resistance remain undetermined. We have recently reported that insulin-induced vasodilation is impaired in afferent arterioles from Zucker obese rats. Furthermore, the myogenic vasoconstriction of this microvessel is diminished.[3] Since the afferent arteriolar tone is recognized to have a buffering action to protect the glomerulus from systemic blood pressure,[21] the impaired myogenic afferent arteriolar vasoconstriction would allow direct transmission of the systemic pressure to the glomerulus. It is therefore anticipated that insulin-resistant animals exhibit glomerular hypertension/hyperfiltration, and consequently, the altered glomerular hemodynamics could participate in part in the development of proteinuria.[20] Alternatively, although it is speculative, the metabolic derangement associated with insulin resistance may directly cause proteinuria. However, the effect of heminephrectomy should be taken into consideration, since heminephrectomy may also elicit glomerular hyperfiltration. In the present study, we have demonstrated that proteinuria develops only in the WFR, not in the WLR, whereas both rat strains undergo heminephrectomy. It is thus strongly suggested that factors associated with a WFR strain, including obesity and insulin resistance, constitute a central determinant of proteinuria. Further studies are required to determine the role of insulin resistance in the progression of renal injury.

Although the present study demonstrates the parallel changes in insulin resistance and proteinuria, it remains undetermined as to whether the obesity per se causes renal injury. It has been demonstrated that in obesity, the increased sympathetic nerve activity contributes to the development of hypertension,[22] which would lead to renal injury. Alternatively, increased sympathetic activity is associated with renal injury.[23] However, in the present study, we found that troglitazone reduced proteinuria without changes in BW (Fig 1). Although it cannot be excluded that troglitazone per se improves proteinuria, it appears unlikely that obesity is responsible for the development of proteinuria and hypertension in this rat strain.

It is widely recognized that insulin-sensitizing agents are potent antidiabetic agents. Recently, troglitazone was demonstrated to bind to peroxisome proliferator–activated receptor gamma and exert its action not only on glucose metabolism but also on other mechanisms independent of insulin sensitization. For example, troglitazone has vasodilator action,[24] which may be associated with vasodepressor activity. Furthermore, troglitazone was recently demonstrated to inhibit the intimal formation of endothelium following balloon injury–induced endothelial damage.[25] These actions on the vascular smooth muscle and endothelium could also affect renal function. Thus, in the present study, we have demonstrated that proteinuria is markedly diminished in troglitazone-treated WFRs (Fig 3). Furthermore, we recently reported that troglitazone restored the impaired myogenic response of renal microvessels in Zucker obese rats.[3] In concert with this, the available evidence strongly

suggests that troglitazone acts as a renal protective agent, and may thus constitute a valuable tool for the treatment of renal injury in insulin resistance. Determining whether troglitazone acts via the correction of insulin resistance or hypertension requires further investigation.

In summary, the present study demonstrates that heminephrectomized WFRs manifest not only insulin resistance but also elevated blood pressure and proteinuria. These alterations are improved by correction of the insulin resistance. However, the earlier onset of the proteinuria versus the hypertension suggests that metabolic alterations per se, rather than hypertension, may contribute to the development of proteinuria. Such metabolic abnormality and its associated conditions in insulin resistance may be factors predisposing to the development of renal injury.

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