Pioglitazone mitigates renal glomerular vascular changes in high-fat, high-calorie-induced type 2 diabetes mellitus


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Pioglitazone mitigates renal glomerular vascular changes in high-fat, high-calorie-induced type 2 diabetes mellitus. Am J Physiol Renal Physiol 291: F694–F701, 2006. First published April 11, 2006; doi:10.1152/ajprenal.00398.2005.—Our hypothesis is that impairment of peroxisome proliferator-activated receptor-γ (PPARγ) initiates renal dysfunction by increasing renal glomerular matrix metalloproteinase-2 (MMP-2) activity because of increased renal homocysteine (Hcy) and decreased nitric oxide (NO) levels. C57BL/6J mice were made diabetic (D) by being fed a high-fat-calorie diet, and an increase in PPARγ activity was induced by adding pioglitazone (Pi) to the diet. Mice were grouped as follows: normal calorie diet (N), D, N+Pi, and D+Pi (n = 6/group). The glomerular filtration rate (GFR), renal artery blood flow and pressure, and plasma glucose were measured. Renal glomeruli and preglomerular arterioles were isolated. Plasma and glomerular levels of NO, Hcy, and MMP activity were measured. The contractile response to phenylephrine and the dilatation response to acetylcholine in renal arteriolar rings were measured in a tissue myograph. In N, D, N+Pi, and D+Pi groups, respectively, GFR was 9.4 ± 1.2, 3.9 ± 1.1, 9.2 ± 1.6, and 8.4 ± 1.4 μl/min·100 g body wt−1. Renovascular resistance was 140 ± 3, 367 ± 21, 161 ± 9, and 153 ± 10 mmHg·ml−1·min−1. Levels of Hcy were increased from 5.8 ± 1.5 in the N to 18.0 ± 4.0 μmol/l in the D group. Glomerular levels of MMP-2 were increased in D mice compared with N mice, and there was no change in levels of MMP-9. Treatment with Pi ameliorated glomerular levels of MMP-2 and Hcy in the D group. Renal artery ring contraction and relaxation by phenylephrine and acetylcholine, respectively, were attenuated in the D group compared with the N groups. Results suggest that a PPARγ agonist ameliorates preglomerular arteriole remodeling in diabetes by decreasing tissue levels of Hcy and MMP-2 activity and increasing NO.

Recent studies from our laboratory in the two-kidney, one-clip mouse model of hypertension demonstrated impaired homocysteine (Hcy) clearance (36). Others have shown a decrease in methylene tetrahydrofolate reductase (15, 30) and CBS enzyme activities in response to increasing insulin and glucose concentrations, leading to hyperhomocysteinemia (5). Renal hyperfiltration early in diabetes without nephropathy was associated with increased Hcy catabolism and clearance (41). Insulin reduces the circulating levels of other amino acids (10) and may promote uptake of Hcy into the tissues, which results in lower plasma Hcy, but increased tissue Hcy (37).

Mild homocysteinemia leads to renal microvascular impairment and vasoconstriction. This, in part, leads to volume retention and further accumulation of Hcy, causing chronic and impaired renal filtration. Although several lines of evidences suggest a strong link between renal disease and accumulation of plasma Hcy late in kidney diseases, there is no report on the levels of glomerular tissue Hcy and renovascular dysfunction.

Although an accumulating body of evidence indicates impaired renal filtration in diabetes, it is unclear, however, whether the impairment is, in part, due to the contractile or relaxation dysfunction in preglomerular blood vessels.

In the normal vessel wall, the matrix metalloproteinases (MMPs) reside in the latent form (35) and are activated during loading (7). During the chronic oxidative atherosclerotic process (11), the endothelium is damaged and the concentration of endothelial nitric oxide (eNO) dwindles (40). To reduce the load, the vessel dilates. However, in the absence of eNO, to dilate the vessel, the latent resident MMP is activated. MMP activation, in turn, degrades elastin as well as ultrastructural collagen (i.e., newly synthesized collagen by proliferating cells). Because the elastin turnover is remarkably lower than collagen (28), the degraded elastin is replaced with stiffer (oxidized) collagen in glomerular microvessels, causing glomerulosclerosis. There is robust MMP activation in diabetes; however, the specific expression of MMP in renal glomeruli is largely unknown. Peroxisome proliferator-activated receptor-γ (PPARγ) agonists have been shown to improve vascular function in diabetes and ameliorate Hcy-mediated endothelial dysfunction (9). We hypothesize that a chronic increase in glomerular Hcy, MMP-2 activation, and a decrease in glomerular nitric oxide (NO) are associated with glomerulosclerosis. PPARγ activation ameliorates glomerulosclerosis and improves metabolic derangements in type 2 diabetes.

METHODS

Diabetic mouse model. Three- to four-week-old male C57BL/6J (B6) mice were obtained from Jackson Labs (Bar Harbor, ME). The C57BL/6J inbred mouse model is highly susceptible to diet-induced type 2 diabetes mellitus, central obesity, atherosclerosis, hyperglycemia, hyperinsulinemia, hyperhomocysteinemia, and hypertension (4, 26, 32, 33). The high-fat diet contains 45% kcal fat, 35% kcal carbohydrate, and 20% kcal protein (Research Diets, New Brunswick, NJ). The normal (10% kcal fat) diet has 10% kcal fat, 70% kcal carbohydrate, and 20% kcal protein. These diets differ in terms of calories; i.e., a high-fat diet contains 4.73 kcal/g, and the normal diet contains 3.85 kcal/g. The high-fat and high-calorie intake disturb the glucose homeostasis and lead to diabetic complications. The mice
were given the following diets for 6 wk and grouped as follows: normal diet (N); normal diet with pioglitazone (Pi; N+Pi); high-fat diet (D); and high-fat diet with Pi (D+Pi).

Mice were fed rodent chow and, to induce PPARγ activation, Pi (Calbiochem) was administered in the food (50 μg Pi/g food). To prevent the diabetic complications, the Pi treatment was started at the same time as the high-fat diet. Based on the fact that mice eat ~4 g food/day, we estimated that each ingested ~200 μg/day of Pi. The binding constant between Pi and PPARγ is in the micromolar range (23). Therefore, dietary consumption of Pi, to produce a blood concentration of ~32 μmol/l, was enough to saturate most binding sites on PPARγ. Others have shown that in humans 100 mg/day of PPARγ agonist has a potent effect (9). Because humans weigh ~75 kg, and mice weigh ~25 g, we estimated that mice ingested approximately sixfold more Pi than did humans. To determine whether Pi treatment caused changes in food intake, food and water intake were measured every 2 days during the treatment period; no changes in intake were found. The mice were killed at 6 wk after the start of the treatment. To determine whether there was peroxisome proliferation, the livers were weighed at the end of the protocol. Animal room temperature was maintained between 22 and 24°C. A 12:12-h light-dark cycle was maintained by artificial illumination. In accordance with National Institute of Health Guidelines for animal research, all animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Louisville School of Medicine.

Direct radiotelemetric measurements of aortic blood pressure. Systolic, diastolic, and mean arterial blood pressure and heart rate were measured continuously during the experiment by a DSI telemetric system (Data Sciences International, St. Paul, MN) using a pressure transducer (PA-C20) surgically implanted into the aorta arch through the left common carotid artery, starting after a 1-wk recovery period. The data were analyzed using DSI Dataquest ART 3.1 software.

Glucose, Hcy, and insulin. At the end of the protocol, plasma glucose and Hcy levels were measured by collecting 1 ml blood in heparinized tubes from anesthetized mice. Glucose was measured using a Bio-Rad glucose measurement kit. Hcy was separated by the Griess method. Total Hcy, protein bound and free, was measured as previously described (37).

Renal arterial trees were carefully dissected up to the interlobular arteries. Mice were fed rodent chow and, to induce PPARγ activation, Pi (Calbiochem) was administered in the food (50 μg Pi/g food). To match the diet intake were found. The mice were killed at 6 wk after the start of the protocol. Animal room temperature was maintained between 22 and 24°C. A 12:12-h light-dark cycle was maintained by artificial illumination. In accordance with National Institute of Health Guidelines for animal research, all animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Louisville School of Medicine.

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Renal glomerular filtration rate. To determine whether plasma levels of Hcy inversely correlate with the renal glomerular filtration rate (GFR), GFR was measured using inulin-FITC as a marker in mice. Plasma levels of inulin-FITC were evaluated from decay over multiple samples from the tail vein. The 24-h urine was collected in metabolic cages. To minimize contamination and variable dryness of the urine, the cages were washed and rinsed, and the collecting tubes were covered in an ice bucket. In all animals, the blood was collected after 24 h of fasting. The level of inulin in blood and urine was measured from a standard plot generated by a spectrofluorometer, using inulin-FITC as the standard, with the emission at 530 nm and excitation at 488 nm. The control plasma and urine were used as references when inulin was measured. The levels of inulin in blood and urine, respectively, of inulin-FITC-injected mice. The excreted inulin (μl·min⁻¹·g body wt⁻¹) was measured from each mouse. To establish steady-state plasma inulin levels over the urine collection period, and to determine mouse-to-mouse variation, plasma inulin levels were measured in each animal. There were no significant mouse-to-mouse differences in plasma inulin levels under these conditions.

Renal histology. The kidneys were stained with trichrome. Glomerular sclerosis was identified by a thickened basement membrane (BM). Theffer arteriolar medial thickness and lumen and outer diameters were measured by a digital micrometer. All histological measurements were undertaken by a person blinded to group allocations.

Total RNA isolation and RT-PCR. Total RNA was isolated from renal arteries of each animal by TRizol (GIBCO). Quantification and purity of the RNA was assessed by 260/280 absorption. Aliquots (2 μg) of total RNA were reverse-transcribed into cDNA using dNTPs plus oligo (dt) primers and SuperScript III reverse transcriptase (Invitrogen). PCR reactions were conducted using the 9600 GeneAmp PCR system (PerkinElmer Life Sciences). Sequence-specific oligonucleotide primers were prepared commercially (Invitrogen). Each PCR was performed in a 12-μl reaction volume containing 1 μl cDNA, 1.2 μl 10X Taq DNA polymerase buffer, 0.2 mM dNTP, 2 mM MgCl2, 100 μM selected primer, and 0.25 U Platinum Taq DNA polymerase buffer. The PCR primers used were as follows: NADPH oxidase (Nox-4): forward 5'-CCCA GAA TGA GGA TCG CAG AA-3'; reverse 5'-TGG AAC TTG GGT TCT TCC AG-3'; GFR, μl·min⁻¹·g BW⁻¹

Table 1. Changes in body weight, plasma insulin, glucose, and glomerular filtration rate at week 1 and week 6 after fat-induced diabetes

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>23±1</td>
<td>25±0.5</td>
</tr>
<tr>
<td>Insulin, pm/l</td>
<td>1.3±0.1</td>
<td>1.35±0.15</td>
</tr>
<tr>
<td>Glucose, mg/l</td>
<td>105±12</td>
<td>110±5</td>
</tr>
<tr>
<td>GFR, μl·min⁻¹·g BW⁻¹</td>
<td>9.6±1.2</td>
<td>9.4±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. BW, body wt; GFR, glomerular filtration rate. *P < 0.05 compared with week 1 in diabetic group; †P < 0.05 compared with week 1 in normal group.
**Table 2. Hemodynamic parameters of diabetic and control mice**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetic</th>
<th>Normal + Pi</th>
<th>Diabetic + Pi</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>25 ± 0.5</td>
<td>40 ± 1</td>
<td>31 ± 0.5</td>
<td>38 ± 1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>MUP, µg·day⁻¹·kg⁻¹</td>
<td>46 ± 5</td>
<td>154 ± 6</td>
<td>47 ± 7</td>
<td>48 ± 9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Liver wt, g</td>
<td>1.89 ± 0.08</td>
<td>1.35 ± 0.07</td>
<td>1.78 ± 0.08</td>
<td>1.91 ± 0.08</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>HW, g</td>
<td>0.13 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>KW, g</td>
<td>0.16 ± 0.01</td>
<td>0.24 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td>0.17 ± 0.02</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>HW/BW×10³</td>
<td>5.2 ± 1.2</td>
<td>4.0 ± 0.8</td>
<td>4.2 ± 1.1</td>
<td>3.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>KW/BW×10³</td>
<td>6.4 ± 1.1</td>
<td>6.0 ± 0.9</td>
<td>6.1 ± 1.2</td>
<td>4.5 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>105 ± 6</td>
<td>122 ± 8</td>
<td>104 ± 4</td>
<td>107 ± 6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>118 ± 6</td>
<td>133 ± 7</td>
<td>116 ± 4</td>
<td>123 ± 6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>84 ± 4</td>
<td>104 ± 3</td>
<td>83 ± 4</td>
<td>87 ± 5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>510 ± 23</td>
<td>580 ± 38</td>
<td>498 ± 32</td>
<td>510 ± 27</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pi, pioglitazone; MUP, mouse urinary protein; HW, heart wt; KW, kidney wt; MAP, mean arterial pressure; SBP and DBP, systolic and diastolic blood pressure, respectively. P values are for diabetic+Pi compared with diabetic groups.

was isolated. The primers for Kim-1 were as described (14, 42): forward 5′-TGT GTG CCT GGT CCA TAG TC-3′; reverse 5′-ATGTCACTTCCCCCATC TTG-3′ and reverse 5′-GAT GCA GGG ATG ATG TTC TG-3′, using the following condition: 94°C for 2 min, followed by 35 cycles of 94°C for 50 s, 55°C for 40 s (Nox-4), 60°C for 60s (eNOS and Kim-1), and 72°C for 1 min, followed by extension at 72°C for 5 min; the primers for GAPDH were forward 5′-ACA ACT TTG GCA TTG TGG AA-3′ and reverse 5′-GAT GCA GGG ATG ATG TTC TG-3′, using the following condition: 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 1 min, followed by final extension at 72°C for 5 min. PCR products were separated on a 1.2% agarose gel and detected under UV transillumination after ethidium bromide staining. The band intensity of the PCR product was analyzed with scanning densitometer software and normalized with GAPDH band intensity.

**Zymographic analysis of MMP activity.** To determine MMP-2 and -9 activities, gelatin substrate gel zymography containing 1% gelatin in 8% SDS-PAGE was performed. The glomerular tissue homogenates were loaded onto the gel with identical amounts of total protein. The bands were scanned using a Bio-Rad GS-700 densitometer with band intensity normalized to β-actin.

**Preparation of phenylephrine and acetylcholine solutions.** The concentrations of acetylcholine (10⁻⁶ to 10⁻⁴ M) and phenylephrine (PE; 10⁻⁶ to 10⁻⁴ M) were based on weight measurements. All dilutions from stock solutions in PBS were made before the experiment. PBS was used as a vehicle control.

**Renal artery function.** Renal artery rings were mounted in a tissue myobath containing PSS maintained at 37°C and bubbled with 95% O₂-5% CO₂. The composition (in mM) of PSS was as follows: 118 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 12.5 NaHCO₃, and 11.1 glucose. The pH of the solution after saturation with 95% O₂-5% CO₂ gas mixture was 7.4. As a routine, tissues were allowed to equilibrate for 1 h before the start of all experiments. One of the two mounted wires (20 µm) was connected to a force transducer.

**Fig. 1.** A and B: plasma levels of glucose and homocysteine (Hcy). Mice were made diabetic (D) by being fed a 45% kcal fat diet. Control (N) mice received a 10% kcal fat diet. Peroxisome proliferator-activated receptor-γ (PPARγ) was induced by pioglitazone (Pi) in the diet for 6 wk. Mice were grouped into normal (N), high-fat diet (D), normal+Pi (N+Pi), and high-fat diet+Pi (D+Pi) groups. Blood was taken from the tail vein. Fasting levels of plasma glucose were measured by a standard kit (test strips). The levels of Hcy were measured by HPLC and spectrophotometry. *P < 0.01 compared with the N group. **P < 0.05 compared with the D group. C: glomerular filtration rate (GFR) was measured in mice. BW, body wt. Before the animals were placed in metabolic cages, 3 mg inulin-FITC/25 g body wt were injected (ip), and 24-h urine was collected. A standard curve was generated using inulin-FITC as a standard and fluorescence was measured at 530 nm when excitation was at 488 nm. Urine inulin-FITC was measured using reference urine from mice that were not given inulin. D: renovascular resistance (RVR) was measured by renal artery blood flow (ml/min) and pressure (mmHg) and expressed as mmHg·ml⁻¹·min⁻¹. *P < 0.05 compared with the N group. **P < 0.05 compared with the D group.

**Values are means ± SE. Pi, pioglitazone; MUP, mouse urinary protein; HW, heart wt; KW, kidney wt; MAP, mean arterial pressure; SBP and DBP, systolic and diastolic blood pressure, respectively. P values are for diabetic+Pi compared with diabetic groups.**
pressure in the D group with no effect on body weight. The liver weight was decreased in the D group and normalized with Pi treatment, suggesting positive effects of PPARγ in diabetes.

**Plasma levels of glucose, Hcy, and GFR.** Plasma glucose levels increased in the D compared with the N group. On the other hand, the D+Pi group did not demonstrate an increased plasma glucose level. Plasma Hcy levels were increased in the D group, and Pi had no effect on the plasma Hcy levels (Fig. 1, A and B). Although there was hyperfiltration early in diabetes (Table 1), there was hypofiltration in D mice after 6 wk of a high-fat diet compared with N mice. Treatment with Pi normalized the GFR in diabetes (Fig. 1C). In addition, there was increase in renal vascular resistance during diabetes. The treatment with Pi normalized renal vascular resistance (Fig. 1D). These results suggest that in chronic type 2 diabetes, there is hypofiltration.

**Renovascular oxidative stress and glomerular injury.** To determine the involvement of NO and reactive oxygen species (ROS) and to conclude that increased NO levels are responsible for the improvement following Pi treatment, we evaluated the NO and ROS generation systems by measuring mRNA expression of eNOS and Nox-4 (NADH oxidase subunit) in preglomerular renal arteries is shown. The expression of the GAPDH gene was used as a control in A. B: mRNA analysis of the expression of kidney injury molecule-1 (Kim-1) in renal glomeruli. The expression of the GAPDH gene was used as a control. Lane 1, ladder of molecular weight markers; lane 2, N+Pi; lane 3, N; lane 4, D; and lane 5, D+Pi groups.

RESULTS

**Hemodynamic and gravimetric parameters.** We made a number of basic measurements at the early vs. late phase of high-fat diet-induced diabetes. The measurements of the changes in body weight, plasma insulin, blood glucose, and GFR were carried out in mice at weeks 1 and 6 of a high-fat diet. The results suggest that diabetic mice have hyperfiltration at an earlier stage, followed by hypofiltration at 6 wk of diabetes (Table 1). There were significant increases in the levels of glucose and insulin in the D groups compared with N groups (Table 1). Although there was no change in body weight with or without Pi treatment in N mice, body weight increased significantly in the D group compared with the N group and was associated with an increase in blood pressure (Tables 1 and 2). The treatment with Pi normalized blood pressure in the D group with no effect on body weight. The liver weight was decreased in the D group and normalized with Pi treatment, suggesting positive effects of PPARγ in diabetes.

**Statistical analysis.** Values are given as means ± SE; n = 6/group. Differences between groups were tested using two-way ANOVA, followed by the Bonferroni post hoc test (34), focusing on the respective effects of diabetes in the N compared with D groups. P < 0.05 was considered significant. P < 0.001 was considered highly significant.

**RESULTS**

**Renovascular oxidative stress and glomerular injury.** To determine the involvement of NO and reactive oxygen species (ROS) and to conclude that increased NO levels are responsible for the improvement following Pi treatment, we evaluated the NO and ROS generation systems by measuring mRNA expression of eNOS and Nox-4 (NADH oxidase subunit), respectively, in preglomerular arterioles. The results suggest that ROS generation was associated with increased Nox-4 expression and a decrease in eNOS expression in the renal vascular system. The treatment with Pi normalized the levels of...
eNOS and Nox-4 in diabetes (Fig. 2). The renal glomerular remodeling was assessed by evaluation of glomerular injury using expression of Kim-1 as an index of more thorough evaluation of renal injury. The results suggest that Kim-1 was highly expressed in glomeruli from diabetic mice. The treatment with Pi mitigated the induction of Kim-1 in diabetes (Fig. 2).

MMP activity in glomeruli. Plasma levels of MMP-2 and MMP-9 activities were higher in the D than in the N group. There appeared to be a constitutive level of MMP-2 and -9 in glomeruli of N mice; however, MMP-2 and -9 were increased in the D group, with a robust increase in MMP-2 activity. These differences were normalized after treatment with Pi (Fig. 3). In zymographic gels (Fig. 3), we showed the active MMP-2 (bottom band). The top latent band was minimal. This suggested that most of the MMP-2 in the diabetic sample was active.

Glomerular tissue levels of Hcy and NO. Glomerular tissue Hcy levels were increased in diabetic mice. The treatment with Pi ameliorated the increase in glomerular tissue Hcy levels. Glomerular levels of NO were decreased in the D group compared with the N group, whereas the glomerular tissue NO levels were normalized with Pi treatment (Fig. 4).

Preglomerular arteriole structure and function. There was significant increase in the media/lumen ratio in diabetic animals compared with normal mice (Fig. 5, A and B). The treatment with Pi normalized this medial thickness. These data complemented the data on increased renal vascular resistance in diabetes. Figure 5, C and D, shows a typical example of the PE response in a diabetic vs. normal vessel. The dose-dependent curves for PE shifted to right in the diabetic group compared with normal mice. The treatment with Pi ameliorated...
the rightward shift in PE dose-response curves in diabetic mice (Fig. 6A). The vascular reactivity measurements suggest an impaired contractile response to PE in diabetic compared with normal mice. The response to acetylcholine was attenuated in diabetic mice compared with control mice. The treatment with Pi normalized this attenuation (Fig. 6B).

Vascular studies tested the contribution of ROS and NO in the high-fat diet-fed mice by measuring vascular reactivity in the presence and absence of Hcy. Because one major action of Hcy is to generate ROS and decrease NO levels, we tested responses to acetylcholine in normal vessels pretreated with Hcy. Also, the connection between Hcy tissue levels and endothelial function was assessed by experiments in which we measured the response to acetylcholine in normal vessels pretreated with Hcy. The results suggest that vessels pretreated with Hcy have a decreased response to acetylcholine (Fig. 6C). This suggests the contribution of Hcy to endothelial dysfunction. Collectively, these results suggest that both the contractile and relaxation impairments contribute to renal dysfunction in diabetes mellitus.

**DISCUSSION**

Although glomerulosclerosis is a hallmark of renal disease, there is no established mechanism as to how glomerulosclerosis is initiated. This study demonstrated that a glomerular-specific increase in MMP-2 activity in diabetes was associated with the accumulation of oxidized matrix and degradation of ultrastructural matrix. The MMP-2 activity was associated with increases in tissue levels of Hcy and decreases in NO, leading to renovascular resistance and impaired renal filtration.

In db/db, type 2 diabetes mice, the accumulation of renal lipid, glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria was associated with the overexpression of steroid-binding proteins (38). Steroid hormones, sex hormone-binding globulin, and Hcy are the markers of lipid and glucose metabolism (1). It has been reported by others that thiazolidinediones reduce plasma Hcy, presumed to increased insulin sensitivity (8). The present study suggests that the increase in plasma glucose in diabetes is linked to an increase in the Hcy level. The treatment with Pi ameliorates the hyperglycemia and has no effect on the level of plasma Hcy. Previously, we showed that Hcy competes with PPARγ ligands, therefore augmenting PPARγ activity, but has no effect on total levels of Hcy (13). In fact, clinical trials with PPAR agonists suggested that treatment with PPAR agonists ameliorated endothelial dysfunction in hyperhomocysteinemia but had no effects on Hcy levels (2). In addition, our results show for the first time that glomerular tissue Hcy plays a significant role in renal impairment. The treatment with Pi mitigates the renal impairment in diabetes.

Accelerated ROS production and diminished bioavailability of NO caused by NOS uncoupling were noted in the diabetic kidney. Administration of tetrahydrobiopterin, a cofactor for eNOS, reversed the decreased dimeric form of eNOS and glomerular NO production (28a). Furthermore, in a nonobese diabetic mouse model, renal hypertrophy and slight glomerular injury in early stages and structural alteration in the proximal straight tubules at later stages during the acute phase of diabetes were attributed to increased neuronal NOS activity (16). Interestingly, we showed an increase in MMP activity in sympathetic nerves in hyperhomocysteinemia (20). In a high-fat-induced type 2 diabetic mouse model, collagen IV deposition in glomerular basement membranes preceded the hyperfiltration and enlargement of glomeruli in the early stage of diabetic nephropathy. The dedifferentiation of mesangial cells was associated with collagen IV deposition (39). There was a robust increase in MMP-2 activity in diabetic glomeruli vs. control. PPARγ ameliorated MMP-2 activation. It has been suggested that diabetes-associated changes in MMP-2 expression are attenuated by Pi treatment, in association with reduced
collagen accumulation and glomerulosclerosis (6). Although several studies (17–19) have suggested expression of MMP in the diabetic kidney, the role of MMP in glomeruli, and more specifically in accumulation of collagen matrix, was not addressed. Because MMP-2 is also an elastase compared with interstitial collagenase (29), it degrades elastin efficiently. Because 50% of the microvascular wall protein is elastin and is responsible for vascular compliance, it is degraded by MMP-2. The turnover of elastin is remarkably slower than collagen, and the degraded elastin is replaced with stiff and oxidized collagen, causing glomerulosclerosis and increasing the media/lumen ratio. Previously, we demonstrated changes in MMP-2 levels that were associated with an accumulation of oxidized matrix and degradation of ultrastructural matrix such as elastin in glomeruli (3). Here, we may suggest a similar mechanism. This may increase vascular stiffness, decreasing vascular contraction and relaxation (3, 12). PPARγ agonists have a specific role in ameliorating the course of progressive tubulointerstitial fibrosis under both normoglycemic and hyperglycemic conditions. The present study suggests that the induction of peroxisome (PPARγ) is associated with decreased levels of MMP-2 activity and amelioration of renal preglomerular arteriolar medial thickness.

The present study suggests three novel findings regarding renal dysfunction with development of type 2 diabetes mellitus. First, this study demonstrated that increases in tissue levels of Hcy are highly accurate in predicting endothelial dysfunction in the diabetic kidney. Also, the increased oxidative stress in diabetes is due, in part, to the increased levels of glomerular Hcy and not plasma Hcy. Second, although numerous studies have suggested impaired renal function and NO levels in type 2 diabetes, there is a lack of information regarding the renovascular resistance and tissue levels of NO in diabetes. Abnormal regulation of endothelial function in diabetes is associated with a decreased contractile response. The decrease in GFR is suggested to be due to impaired relaxation, but the impaired constriction would do just the opposite to GFR. The impaired constriction may not be consistent with a decrease in GFR. The acetylcholine-induced dilitation was attenuated in the diabetic kidney. This study suggests that the renal arterioles may have both a contractile as well as a relaxation dysfunction in high-calorie-induced type 2 diabetes. Finally, in ex vivo experiments, the data suggest amelioration of impaired renal arteriole contraction and relaxation in diabetes by a PPARγ agonist.

Hcy and NO data may be more likely to be associative and secondary to alterations in insulin resistance or other metabolic changes. The addition of Pi may well vary the inulin excretion activity and amelioration of renal dysfunction in the early stages of obesity. These studies are in progress. Additional experiments to directly test the associations of oxidative stress and MMP in vascular dysfunction will require study using antioxidants and MMP blockers. These studies are in progress.

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