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ORIGINAL ARTICLE

Activation of Peroxisome Proliferator–Activated Receptor δ Inhibits Streptozotocin-Induced Diabetic Nephropathy Through Anti-Inflammatory Mechanisms in Mice

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OBJECTIVE—Activation of the nuclear hormone receptor peroxisome proliferator–activated receptor (PPAR)-δ has been shown to improve insulin resistance, adiposity, and plasma HDL levels. Several studies have reported that activation of PPARδ is atheroprotective; however, the role of PPARδ in renal function remains unclear. Here, we report the renoprotective effects of PPARδ activation in a model of streptozotocin-induced diabetic nephropathy.

RESEARCH DESIGN AND METHODS—Eight-week-old male C57BL/6 mice were divided into three groups: *1*) nondiabetic control mice, *2*) diabetic mice, and *3*) diabetic mice treated with the PPARδ agonist GW0742 (1 mg/kg/day). GW0742 was administered by gavage for 8 weeks after inducing diabetes.

RESULTS—GW0742 decreased urinary albumin excretion without altering blood glucose levels. Macrophage infiltration, mesangial matrix accumulation, and type IV collagen deposition were substantially attenuated by GW0742. The gene expression of inflammatory mediators in the kidney cortex, such as monocyte chemoattractant protein-1 (MCP-1) and osteopontin (OPN), was also suppressed. In vitro studies demonstrated that PPARδ activation increased the expression of anti-inflammatory corepressor B-cell lymphoma-6, which subsequently suppressed MCP-1 and OPN expression.

CONCLUSIONS—These findings uncover a previously unrecognized mechanism for the renoprotective effects of PPARδ agonists and support the concept that PPARδ agonists may offer a novel therapeutic approach for the treatment of diabetic nephropathy. ***Diabetes*** **60:960–968, 2011**

The increasing prevalence of diabetic nephropathy worldwide is a major societal issue because of the enormous expense associated with kidney replacement therapy (1). The pathogenesis of diabetic nephropathy is complex and involves multiple pathways that lead to kidney injury, including the polyol pathway (2), protein kinase C (3), advanced glycation end products (4), and transforming growth factor (TGF)-β (5). In addition, inflammation is now recognized to play an important role in the development of diabetic nephropathy (6,7). In this condition, the accumulation of macrophages and increased expression of cell adhesion molecules are observed in renal biopsy specimens from patients with diabetic nephropathy (8). We have demonstrated that inflammation in diabetic nephropathy can be ameliorated by inhibiting macrophage infiltration in intercellular adhesion molecule-1 (ICAM-1) and macrophage scavenger receptor-A (SR-A) knockout mice (9,10). Therefore, inflammation could be a major therapeutic target of diabetic nephropathy.

There is an increasing body of evidence suggesting that a subfamily of nuclear hormone receptor transcription factors, namely the peroxisome proliferator–activated receptors (PPARs) (PPARγ, PPARα, and PPARδ), may play important roles in the pathogenesis of metabolic syndrome, obesity, and diabetes (11). PPARs are also implicated in many renal pathophysiological conditions, including diabetic nephropathy and glomerulosclerosis (12). Synthetic PPARγ and PPARα agonists, such as thiazolidinediones and fibrates, improve the glycemic control in type 2 diabetic patients and lower the serum triglyceride levels in hyperlipidemic patients. In addition, we and other investigators have reported that PPARγ and PPARα agonists are also beneficial in diabetic nephropathy (13–15). Although atheroprotective effects of PPARδ agonists have been reported (16,17), there are no reports regarding the effects of PPARδ agonists on diabetic nephropathy.

The purpose of the current study was to investigate the hypothesis that activation of PPARδ prevents the development of diabetic nephropathy by inhibiting inflammatory processes, including chemokine/cytokine expression and macrophage infiltration.

RESEARCH DESIGN AND METHODS

Experimental protocol. Male C57BL/6J mice were purchased from Charles River (Yokohama, Japan). Eight-week-old mice were divided into three groups: *1*) nondiabetic control mice (control; $n = 6$), *2*) streptozotocin (STZ)–induced diabetic mice (DM; $n = 7$), and *3*) diabetic mice treated with PPARδ agonist GW0742 (DM+GW0742; $n = 7$). GW0742 mice were purchased from Sigma-Aldrich (Tokyo, Japan). Diabetes was induced by peritoneal injection of 200 mg/kg STZ (Sigma-Aldrich) in citrate buffer (pH 4.5). Blood glucose was measured by the glucose oxidase method at 3 and 7 days after STZ injection, and only mice with blood glucose concentrations >16 mmol/L were used in the study. Mice in the control group were injected with citrate buffer. The DM+GW0742 group received 1 mg/kg/day of GW0742 by gavage for 8 weeks. All mice had free access to standard diet and tap water. All procedures were performed according to the Guidelines for Animal Experiments at Okayama University Medical School, Japanese Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). Mice were killed at 8 weeks after

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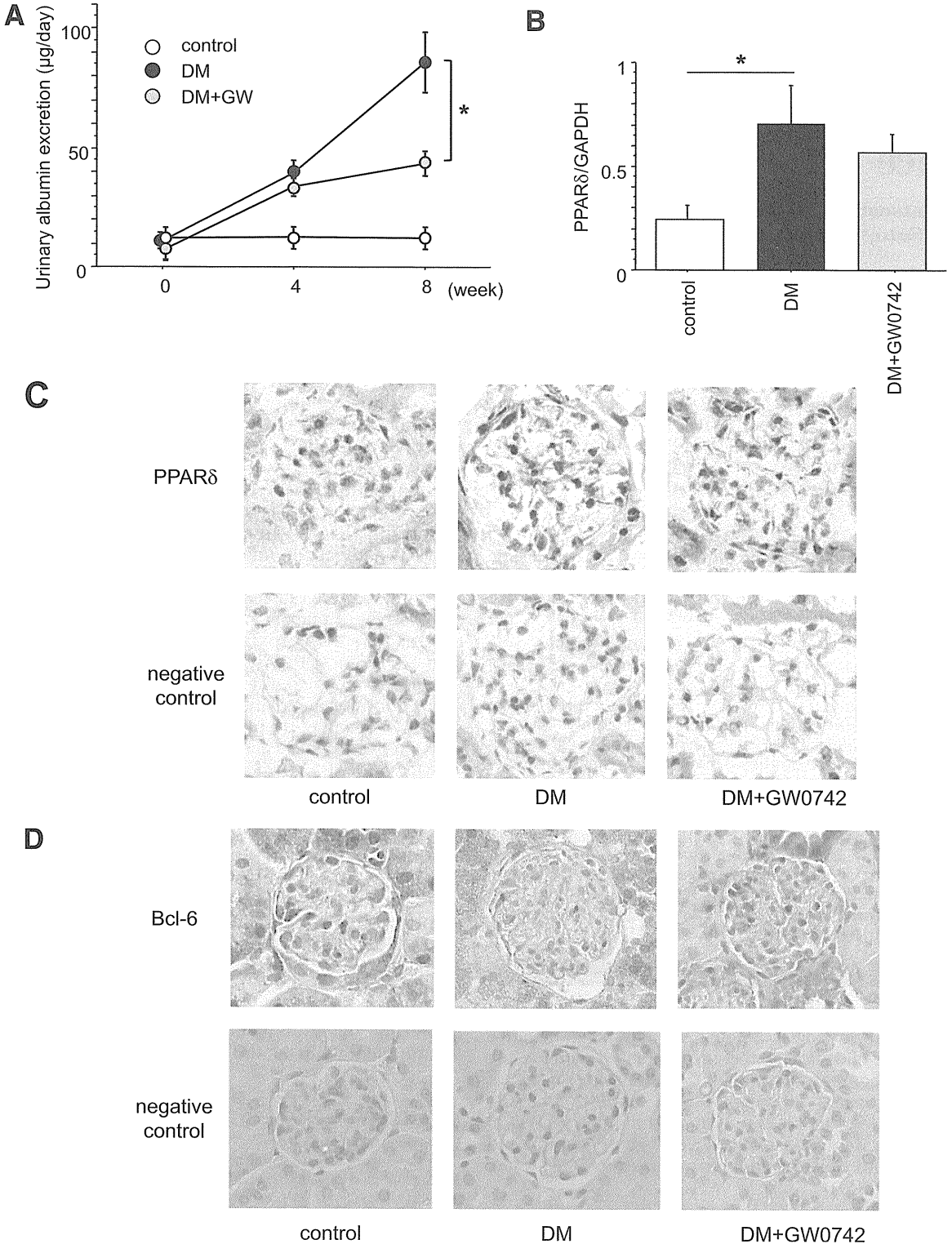

FIG. 1. Time course of changes in UAE and PPARδ mRNA and protein expression in the kidneys. *A*: The UAE increased progressively in the untreated diabetic (DM) group during the 8-week observation period after the induction of diabetes. GW0742 treatment (DM+GW0742) significantly reduced UAE at 8 weeks compared with the DM group. Control, nondiabetic control mice. Data are means ± SE. *$P < 0.01$ for DM vs. DM+GW0742. *B*: Renal PPARδ mRNA expression was significantly increased in the DM group compared with the control group. Data are means ± SE. *$P < 0.05$. *C*: Localization of renal PPARδ protein expression by immunohistochemistry. PPARδ protein expression was predominantly localized in the glomeruli of the DM and DM+GW0742 groups. Original magnification ×400. *D*: Localization of renal Bcl-6 protein expression by immunohistochemistry. Bcl-6 protein was mainly expressed in the glomeruli of the control group, but its expression was suppressed in the DM group. The expression of Bcl-6 recovered in the DM+GW0742 groups compared with the DM group. Original magnification ×400. (A high-quality digital representation of this figure is available in the online issue.)

inducing diabetes. The kidneys were removed, weighed, and fixed in 10% formalin for periodic acid–methenamine silver (PAM) staining, and parts of the remaining tissues were embedded in optimal cutting temperature compound (Sakura Finetechnical, Tokyo, Japan) and frozen immediately in acetone cooled on dry ice. Other tissues were snap-frozen in liquid nitrogen and stored at −80°C.

Metabolic data. We measured body weight, blood pressure, hemoglobin A_{1c} (HbA_{1c}), 24-h urinary albumin excretion (UAE), and creatinine clearance at 0, 4, and 8 weeks. Blood pressure was measured using the tail-cuff method (Softron, Tokyo, Japan). HbA_{1c} was measured using the high-pressure liquid chromatography method, and serum creatinine was measured using the enzymatic method. Urine was collected for 24 h, with each mouse individually housed in a metabolic cage and provided with food and water ad libitum. Urinary albumin concentration was measured as previously described (9). Creatinine was measured enzymatically, and creatinine clearance was calculated.

Light microscopy. PAM-stained sections were analyzed. To evaluate glomerular size, we examined 10 randomly selected glomeruli in the cortex per animal under high magnification (×400) at 8 weeks after induction of diabetes. The area of the glomerular tuft and the mesangial matrix index (MMI) were measured using Lumina Vision software (Mitani Corporation, Tokyo, Japan). MMI was defined as the PAM-positive area in the tuft area, calculated using the following formula: MMI = (PAM positive area)/(tuft area). The results are expressed as means ± SE (per μm^2 for tuft area; arbitrary units for MMI).

Immunoperoxidase staining. Immunoperoxidase staining was performed as previously described (9). Briefly, fresh frozen sections were cut to 4 μm thick using a cryostat. To evaluate macrophage infiltration, we applied a rat anti-mouse monocyte/macrophage (F4/80) monoclonal antibody (Abcam, Tokyo, Japan), followed by biotin-labeled goat antirat IgG antibody (Jackson Immunoresearch Laboratories, West Grove, PA). The avidin-biotin coupling reaction was performed on sections using a Vectastain Elite kit (Vector Laboratories, Burlingame, CA). We examined 10 glomeruli per animal and counted the number of F4/80-positive cells. The mean number of positive cells per glomerulus and interstitial tissue (number per mm^2) were used for the estimation. To evaluate PPARδ and Bcl-6 expression, PPARδ rabbit polyclonal antibody (Affinity BioReagents, Golden, CO) and Bcl-6 rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) were applied, followed by biotin-labeled donkey anti-rabbit IgG antibody (Jackson Immunoresearch Laboratories).

Immunofluorescent staining. Immunofluorescent staining was performed as previously described (9). To clarify the differences in mesangial matrix proteins, we used rabbit anti-type IV collagen antibody (Millipore, Temecula, CA), followed by Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen, Carlsbad, CA). Fluorescence pictures were obtained using a fluorescence microscope (BX51; Olympus, Tokyo, Japan). The type IV collagen immunofluorescence intensity was quantified as previously described (9). Briefly, using Lumina Vision software (Mitani Corporation), the intensity of expression on the images was calculated using the formula, x (density) × positive area (μm^2). The positive area of type IV collagen in each glomerulus was estimated as the ratio to the mean area of the glomerulus. Ten glomeruli per animal were evaluated.

Quantitative analysis of renal cortex gene expression. The RNA from the renal cortex was isolated 8 weeks after treatment using an RNeasy Mini Kit (Qiagen, Valencia, CA). Single-strand cDNA was synthesized from the extracted RNA using a RT-PCR kit (Perkin Elmer, Foster City, CA). To evaluate the mRNA expression of PPARδ, CD14, CD11c, monocyte chemoattractant protein (MCP)-1, chemokine CC motif receptor 2 (CCR2), TGF-β, tumor necrosis factor (TNF)-α, osteopontin (OPN), and ICAM-1 in the renal cortex, quantitative RT-PCR (qRT-PCR) was performed using StepOnePlus (Applied Biosystems, Tokyo, Japan) and FastStart SYBR Premix Ex Taq II (Takara Bio, Otsu, Japan). The primers were purchased from Takara Bio. Each sample was analyzed in triplicate and normalized for GAPDH mRNA expression.

Cell culture and treatment. RAW 264.7 murine macrophages were cultured in Dulbecco's modified Eagle's medium supplemented with 1,000 mg/L D-glucose, 10% FBS, 100 units/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L L-glutamine. For ligand treatment, cells were serum-starved by culture in 0.5% FBS for 24 h. After pretreatment with GW0742 (10 μmol/L) for 24 h, the cells were stimulated with 4,500 mg/L D-glucose (high glucose) for 24 h. Individual experiments were repeated at least three times with different lots or preparation of cells.

Quantitative analysis of gene expression in RAW macrophages. Total RNA was prepared from cells using an RNeasy Mini Kit (Qiagen) as described above. B-cell lymphoma-6 (Bcl-6), MCP-1, and OPN mRNA expression in RAW macrophages was measured using qRT-PCR, as described above.

Immunoprecipitation and Western blotting. Bcl-6 and PPARδ protein expression levels were determined by Western blotting. To examine the interactions between PPARδ and Bcl-6, the nuclear protein fraction was isolated from RAW macrophages using a Nuclear Extract Kit (Active Motif, Carlsbad, CA). The nuclear protein was immunoprecipitated with an anti-PPARδ antibody (Affinity BioReagents) for 1.5 h at 4°C. The nuclear protein–antibody complex was then incubated with magnetized Protein G Dynabeads (Invitrogen) for 45 min at room temperature. After washing the beads, the bound proteins were eluted and resolved by SDS-PAGE. Protein was transferred to nitrocellulose membranes and blocked in 20 mmol/L Tris-HCl (pH 7.6) containing 150 mmol/L NaCl, 0.1% Tween-20, and 5% (wt/vol) nonfat dried milk. The blots were then incubated with anti-PPARδ antibody (Affinity BioReagents) and anti–Bcl-6 antibody (Santa Cruz Biotechnology). The immunoblots were hybridized with anti-TATA binding protein antibody (Abcam) to monitor equivalent loading in different lanes. All experiments were repeated at least three times.

Statistical analysis. All values are means ± SE. Statistically significant differences between groups were examined using one-way ANOVA followed by Scheffé test. A P value <0.05 was considered statistically significant.

RESULTS

Metabolic data and time course of changes in UAE. The UAE progressively increased in diabetic mice during the study (Fig. 1*A*). However, GW0742 treatment significantly reduced the mean UAE (86.09 ± 12.67 μg/day) compared with the DM group at 8 weeks after inducing diabetes (40.91 ± 3.94 μg/day; $P < 0.01$). The other metabolic data are summarized in Table 1. Eight weeks after inducing diabetes, there were no significant differences in systolic blood pressure between the three groups. HbA_{1c}, kidney weight, and relative kidney weight were significantly higher in the DM group than in the control group. There was no significant difference in HbA_{1c}, kidney weight, and relative kidney weight between the DM and the DM+GW0742 groups. Body weight was lower in both the DM and the DM+GW0742 groups than in the control, but was higher in the DM+GW0742 than in the DM group. There were no significant differences in creatinine clearance or triglyceride levels between the three groups.

Renal PPARδ and Bcl-6 expression. We found PPARδ mRNA and protein expression in the kidneys. At 8 weeks,

TABLE 1
Metabolic data at 8 weeks after inducing diabetes

	Control	DM	DM+GW0742
n	6	7	7
Systolic blood pressure (mmHg)	97.8 ± 7.9	111.8 ± 4.4	110.1 ± 3.5
HbA_{1c} (%)	4.60 ± 0.04	9.75 ± 0.34*	8.81 ± 0.62*
Body weight (g)	26.75 ± 0.39	17.74 ± 1.03*	20.70 ± 0.41*†
Kidney weight (mg)	283.3 ± 3.3	325.7 ± 18.8‡	295.7 ± 6.1
Relative kidney weight (mg/g body wt)	11.82 ± 0.27	18.85 ± 1.87*	15.87 ± 0.57
Creatinine clearance (mL/min)	249.0 ± 34.8	349.5 ± 40.4	348.1 ± 44.7
Triglycerides (mg/dL)	23.6 ± 2.0	27.9 ± 7.3	21.5 ± 4.8

Data are means ± SE. *$P < 0.01$ vs. the control group. †$P < 0.01$ vs. the DM group. ‡$P < 0.05$ vs. the control group.

renal PPARδ mRNA expression was significantly greater in the DM group than in the control group (0.71 ± 0.18 vs. 0.24 ± 0.07, respectively; $P < 0.05$) (Fig. 1*B*). However, GW0742 treatment did not affect PPARδ mRNA expression in renal tissues. Renal sections immunostained with PPARδ-specific antibodies revealed that PPARδ protein expression was predominantly localized in the glomeruli of DM and DM+GW0742 groups and to a lesser extent in the glomeruli of the control group (Fig. 1*C*). By contrast, the anti-inflammatory corepressor Bcl-6 was mainly expressed in the glomeruli of the control group, and its expression was suppressed in the DM group. GW0742 treatment recovered the expression of Bcl-6 compared with the DM group (Fig. 1*D*).

MMI and expression of type IV collagens in the glomeruli. Representative glomeruli in PAM-stained sections are shown in Fig. 2*A*. Glomerular hypertrophy and mesangial matrix expansion were observed in the DM group at the end of the 8-week observation period. However, these changes were ameliorated in the DM+GW0742 group compared with DM group (MMI: 9.05 ± 0.30 vs. 12.34 ± 0.49%, respectively; $P < 0.001$) (Fig. 2*B*). A similar trend was noted for type IV collagen (Fig. 2*C*). The type IV collagen–positive area in glomeruli was larger in the DM group than in the control group. This area was markedly reduced in the DM+GW0742 group compared with the DM group (9.57 ± 0.18 vs. 12.33 ± 0.49%, respectively; $P < 0.001$) (Fig. 2*D*).

Macrophage infiltration in the kidney. The number of macrophages in the glomeruli was remarkably higher in the DM group than in the control group. Interestingly, macrophage infiltration into the glomeruli was significantly reduced in the DM+GW0742 group compared with the DM group (1.80 ± 0.08 vs. 2.69 ± 0.05, respectively; $P < 0.001$) (Fig. 3*A* and *B*). Similarly, macrophage infiltration into the interstitium was increased in the DM group but was suppressed in the DM+GW0742 group (13.79 ± 0.53 vs. 7.75 ± 0.77, respectively; $P < 0.001$) (Fig. 3*A* and *C*).

Macrophage and inflammatory gene expression in the renal cortex. qRT-PCR analyses of kidney tissue demonstrated that the expression of two macrophage marker genes, *CD14* and *CD11c*, was increased in the DM group and that GW0742 treatment markedly reduced the expression of these genes (Fig. 4). *CD14* is a marker for all macrophages, and *CD11c* is specific for the M1 subtype of macrophages. Similarly, the induction of diabetes increased the renal expression of several proinflammatory and proatherogenic genes, including *MCP-1*, *TGF-β*, *OPN*, *TNF-α*, and *ICAM-1*. Although GW0742 decreased the expression of *MCP-1*, *TGF-β*, and *OPN*, it did not affect *TNF-α* or *ICAM-1* (Fig. 4). Of note, *MCP-1*, a key chemokine involved in macrophage recruitment, plays a significant role in diabetic nephropathy because the absence of *MCP-1* significantly reduces diabetic renal injury (18,19). Similarly, *OPN* is also a critical inflammatory cytokine involved in diabetic nephropathy (20,21). Collectively, these data indicate that the PPARδ agonist GW0742 inhibits diabetes–induced macrophage recruitment and inflammatory gene expression in the kidney.

PPARδ and Bcl-6 expression in RAW macrophages. PPARδ has been demonstrated to directly affect the anti-inflammatory transcriptional repressor Bcl-6. PPARδ agonists increase free Bcl-6 in macrophages, suppressing MCP-1 transcription and decreasing macrophage infiltration (22). To investigate the regulation of renal inflammatory pathways by diabetes and PPARδ, and the roles of Bcl-6 and PPARδ in these processes, we performed in vitro

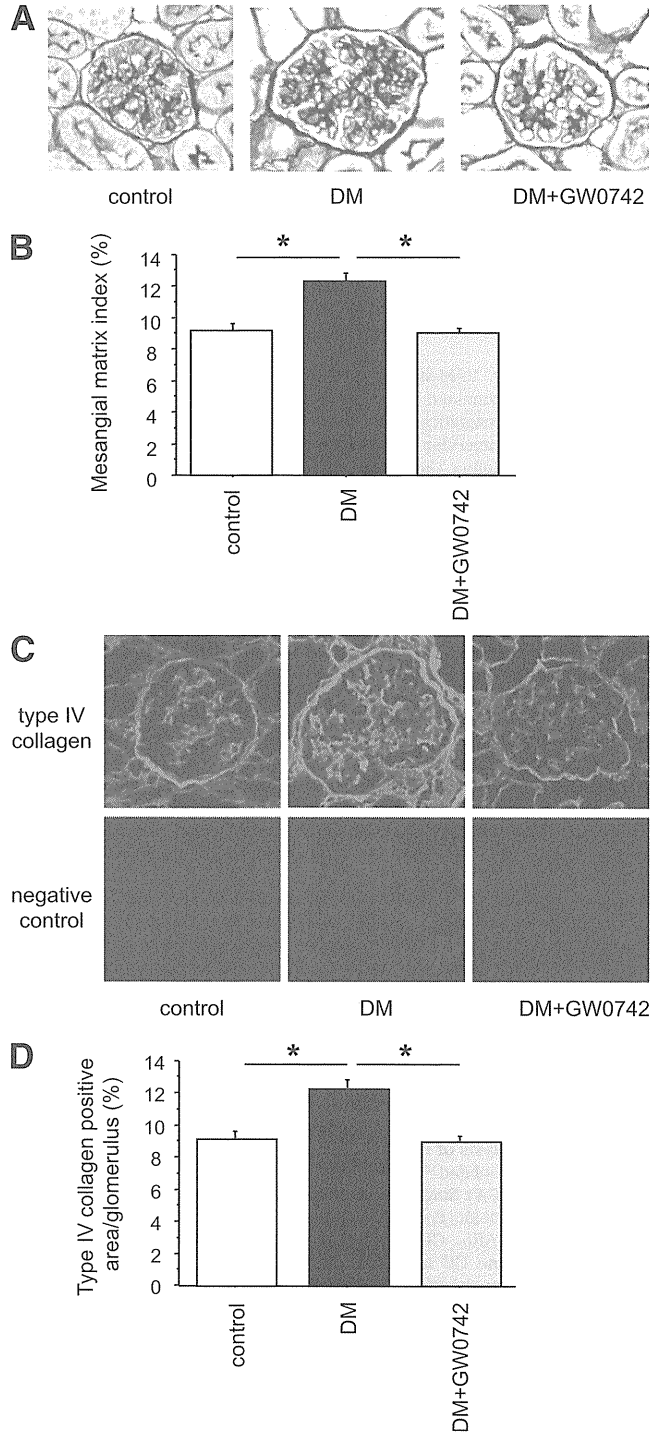

FIG. 2. PAM staining of kidney sections and the expression of type IV collagen in the kidney. *A*: Representative glomeruli from control, DM, and DM+GW0742 mice. Glomerular hypertrophy and mesangial matrix expansion were evident in the DM group. GW0742 suppressed the increase in the MMI compared with the DM group. Original magnification ×400. *B*: MMI in glomeruli. Data are means ± SE. *$P < 0.001$. *C*: Type IV collagen was significantly increased in the DM group compared with the control group and was decreased in the DM+GW0742 group compared with the DM group. Original magnification ×400. *D*: Type IV collagen–positive area in glomeruli. Data are means ± SE. *$P < 0.001$. (A high-quality digital representation of this figure is available in the online issue.)

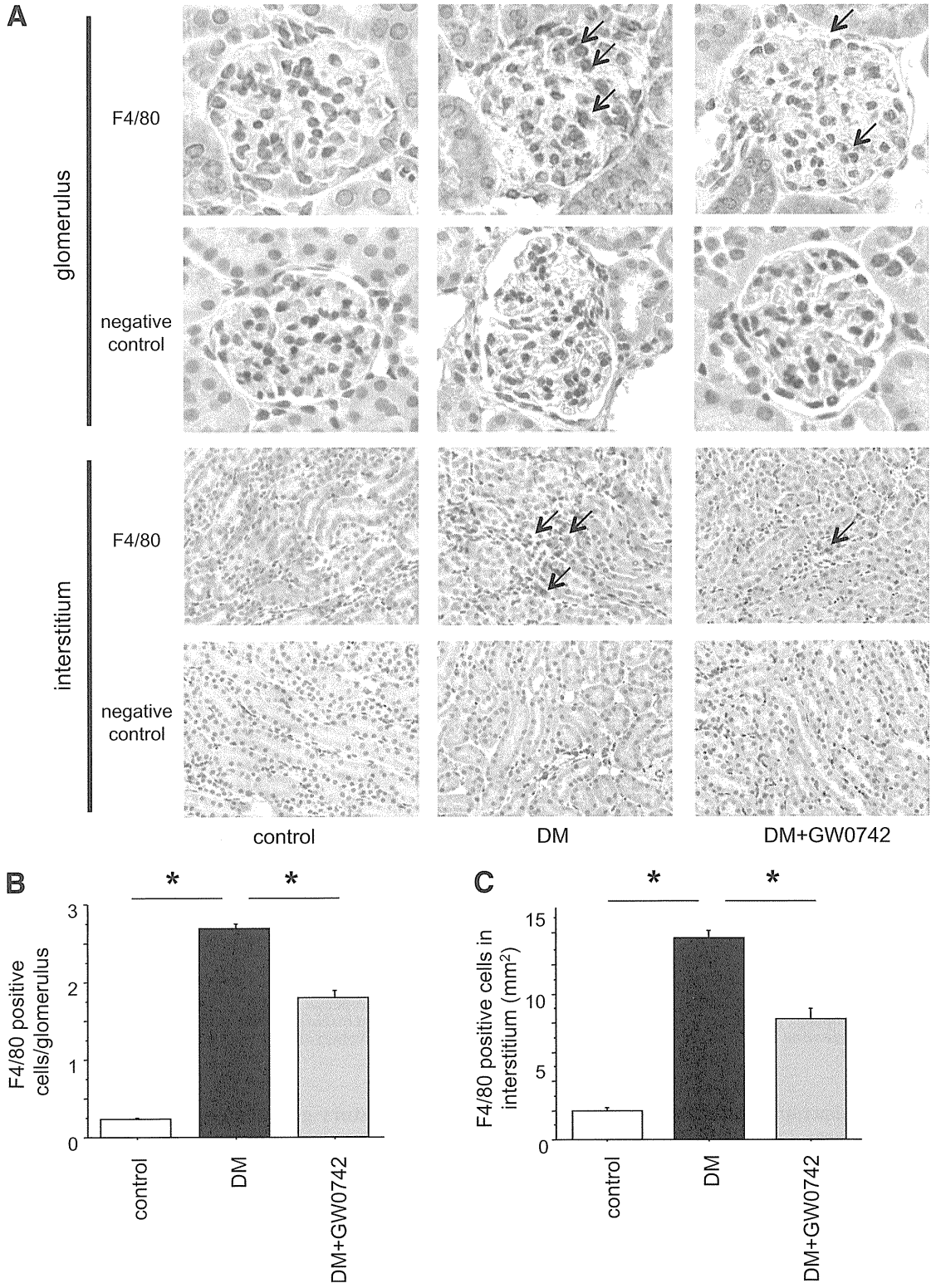

FIG. 3. Macrophage infiltration into the kidney. *A*: Macrophage (*arrows*) infiltration into glomeruli and interstitium was remarkable in the DM group and was suppressed in the DM+GW0742 group. Original magnification ×400. *B*: The number of intraglomerular macrophages. Data are means ± SE. *$P < 0.001$. *C*: The number of macrophages in interstitium. Data are means ± SE. *$P < 0.001$. (A high-quality digital representation of this figure is available in the online issue.)

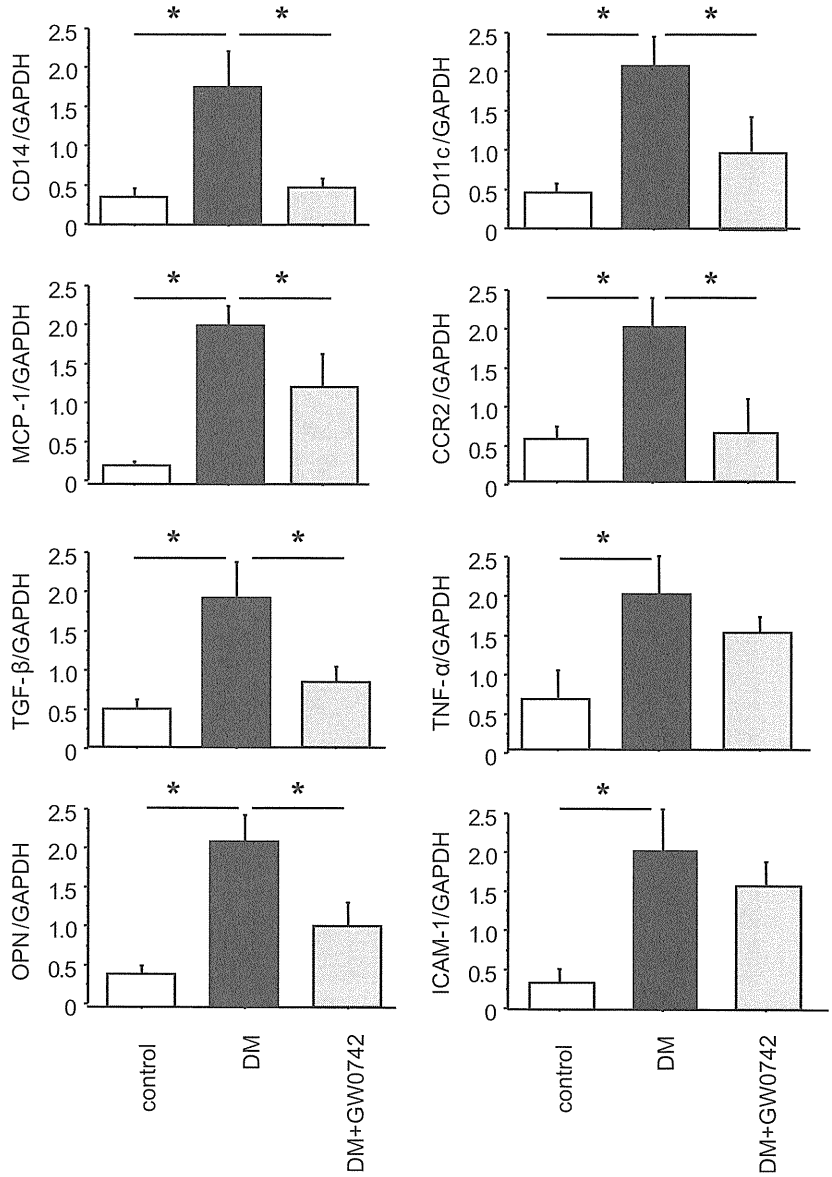

FIG. 4. PPARδ activation suppresses diabetes–induced renal inflammation and macrophage infiltration. Quantitative RT-PCR analysis of the expression of two macrophage markers (CD14 and CD11c) shows that GW0742 inhibited diabetes–induced macrophage infiltration into the kidney. Similarly, GW0742 suppressed MCP-1, CCR-2, TGF-β, and OPN mRNA levels in the kidney. mRNA levels are normalized to GAPDH. Data are means ± SE. *$P < 0.05$.

studies using RAW macrophages. qRT-PCR analyses revealed that the high-glucose medium strongly inhibited Bcl-6 expression in RAW macrophages and that GW0742 treatment significantly attenuated this inhibition in macrophages (Fig. 5*A*). We found that macrophages exposed to high glucose had an increase in nuclear PPARδ protein and that pretreatment with GW0742 completely abolished this effect (Fig. 5*B*). Western blot analyses of total and PPARδ-bound Bcl-6 in macrophage nuclear extracts revealed that high glucose tended to suppress total Bcl-6 but markedly increased PPARδ–Bcl-6 complexes and that GW0742 pretreatment decreased PPARδ–Bcl-6 binding (Fig. 5*C*). These data indicate that high glucose can increase nuclear PPARδ–Bcl-6 binding, limiting the amount of free Bcl-6 available to repress the expression of inflammatory genes normally suppressed by Bcl-6.

Inflammatory gene expression in RAW macrophages. To further investigate the role of PPARδ activation in inflammatory processes, we examined the effects of GW0742 on inflammatory gene expression in macrophages. Consistent with the changes in free Bcl-6, high glucose–stimulated MCP-1 expression, which is regulated by Bcl-6, was attenuated by GW0742 (Fig. 6*A*). The expression of OPN, a macrophage chemokine, was also increased by exposure to high glucose and suppressed by GW0742 (Fig. 6*B*).

DISCUSSION

In the current study, we demonstrated that the PPARδ agonist GW0742 ameliorated albuminuria, glomerular mesangial expansion, and type IV collagen accumulation without affecting blood glucose levels in STZ–induced

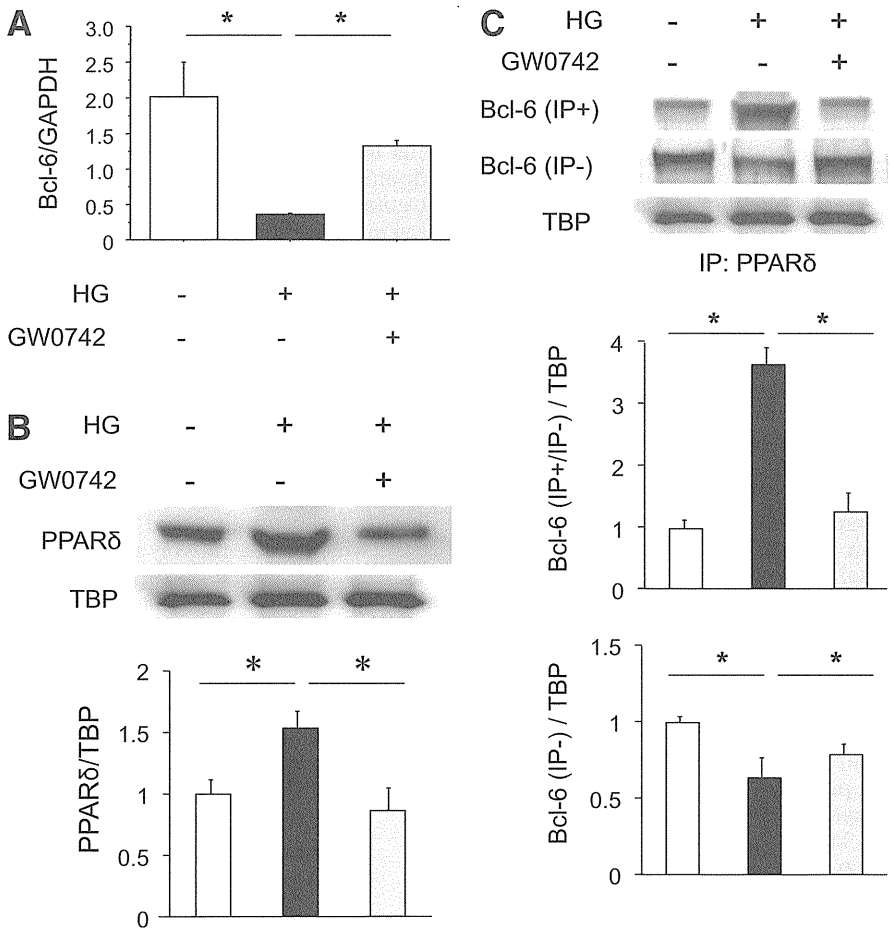

FIG. 5. High glucose suppresses Bcl-6 expression in RAW macrophages. *A*: mRNA isolated from macrophages was analyzed by quantitative RT-PCR. Bcl-6 expression was normalized to GAPDH. Data are means ± SE. *$P < 0.05$. *B*: High glucose increases and GW0742 decreases macrophage PPARδ protein expression. PPARδ expression was normalized to that of TBP. Data are means ± SE. *$P < 0.05$. *C*: Bcl-6 protein expression and PPARδ–Bcl-6 interaction in macrophages in response to high glucose and/or GW0742. The Bcl-6–PPARδ interaction was analyzed by Western blotting of total and PPARδ-bound Bcl-6 in macrophage nuclear proteins after pull-down assays. Bcl-6 expression was normalized to that of TBP. Data are means ± SE. *$P < 0.05$.

diabetic mice. GW0742 treatment markedly decreased macrophage infiltration in diabetic kidney and the expression of inflammatory genes, including *MCP-1*, *TGF-β*, and *OPN*. Furthermore, in vitro studies with RAW macrophages revealed that high glucose suppressed the expression of free Bcl-6, which was associated with increased expression of MCP-1 and OPN, and that GW0742 reversed these effects. Our findings suggest that the activation of PPARδ

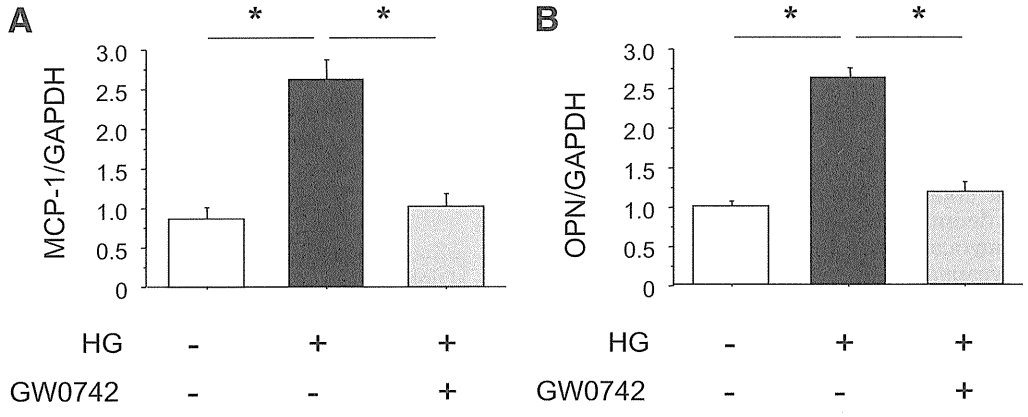

FIG. 6. GW0742 suppresses MCP-1 (*A*) and OPN (*B*) mRNA expression in RAW macrophages. mRNA isolated from macrophages was analyzed by quantitative RT-PCR. MCP-1 and OPN expression was normalized to GAPDH. Data are means ± SE. *$P < 0.05$.

has an anti-inflammatory effect in diabetic kidneys and prevents the development of nephropathy, independently of blood glucose levels.

The nuclear receptor transcription factors PPARγ, PPARα, and PPARδ are critical in regulating insulin sensitivity, adipogenesis, lipid metabolism, and inflammation (11). All three PPAR isoforms have been proposed as therapeutic targets for the treatment of metabolic syndrome, dyslipidemia, and diabetes (11). Several recent clinical studies have provided evidence that thiazolidinedione, PPARγ agonists, and fibrates, PPARα agonists, confer a renoprotective effect in patients with type 2 diabetes (23–25). Experimental studies have also shown beneficial effects of PPARγ agonists on renal injury in type 1 and type 2 diabetic animal models, and multiple mechanisms seem to be involved (26,27). We previously demonstrated that the PPARγ agonist pioglitazone ameliorates diabetic nephropathy by inhibiting cell cycle–dependent hypertrophy of podocytes by reducing Bcl-2 and p27Kip1 protein levels (13) and by suppressing ICAM-1 expression and macrophage infiltration by inhibiting nuclear factor-κB activation in endothelial cells (14). PPARα agonists also have renoprotective effects by reducing TGF-β and plasminogen activator inhibitor-1 expression in mesangial cells (15). However, unlike PPARγ and PPARα, little is known about the therapeutic potential of PPARδ agonists in diabetic nephropathy.

Many studies have proposed an important role of inflammatory processes in the pathogenesis of diabetic nephropathy (6,7). We previously reported macrophage infiltration and increased expression of leukocyte adhesion molecules in the kidneys of patients with diabetic nephropathy in addition to mesangial matrix expansion and interstitial fibrosis (8). We have also described the importance of ICAM-1–dependent infiltration of macrophages into the kidney in the pathogenesis of diabetic nephropathy in a series of studies (9,28). Furthermore, we have demonstrated that SR-A–deficient mice are protected from renal injury after induction of diabetes by inhibiting macrophage migration into diabetic kidneys (10). Other studies have reported that the chemokine MCP-1 plays an important role in the pathogenesis of diabetic nephropathy (29). The importance of MCP-1 in the early development of diabetic nephropathy has been determined using animal models incorporating genetically deficient mice or therapeutic blockade of the MCP-1 receptor CCR2 (18,19,30). In the current study, MCP-1 expression in the kidney was increased in diabetic mice and suppressed by the administration of GW0742. PPARδ activation has been suggested to reduce MCP-1 and subsequently reduces the number of recruited macrophages in diabetic glomeruli and interstitium.

In this study, we found that diabetes significantly increased PPARδ expression in the kidney, and that high glucose increased PPARδ expression in cultured RAW macrophages. In contrast to our observations, Yu et al. (31) reported a decrease in PPARδ gene and protein expression in the myocardium of diabetic rats. On the other hand, several investigators observed an increase in PPARδ expression in the lung (32), diaphragm (33), and aorta (16) in diabetic animals. Taken together, these results suggest that PPARδ expression in the diabetic state differs between individual tissues and may depend on the balance between the two major fuel utilization pathways (i.e., glucose vs. fatty acid metabolism and their cross-talk) (33). The mechanisms by which high glucose increases the expression of PPARδ remain unclear, and further studies are needed.

Unliganded PPARδ, which binds to the anti-inflammatory transcriptional repressor Bcl-6, reduces the amount of free Bcl-6 available to suppress MCP-1 transcription, thus increasing MCP-1 expression (22). We showed that administration of the PPARδ agonist GW0742 substantially attenuated STZ–induced diabetic nephropathy without altering the blood glucose level and increased the renal expression of Bcl-6, which was associated with suppression of *MCP-1* gene expression in the kidney. We have searched for the PPRE in the promoter region of Bcl-6, but have been unable to locate any PPRE sites. Thus, we speculate that activation of PPARδ may induce other transcriptional factors, which induce *Bcl-6* gene expression. Moreover, immunoprecipitation and Western blotting revealed that high glucose increased the PPARδ–Bcl-6 complex, reducing the amount of free Bcl-6 in cultured macrophages. Activation of PPARδ by GW0742 decreased PPARδ–Bcl-6 binding and increased the amount of free Bcl-6, which consecutively decreased MCP-1 expression in macrophages. Based on these in vivo and in vitro data, inhibition of MCP-1 expression by PPARδ agonists is likely mediated through increased expression of Bcl-6 (Fig. 7).

OPN acts as a chemokine and as a proinflammatory cytokine (34). We previously demonstrated that OPN expression was increased in diabetic kidneys and suppressed by SR-A deficiency (10). Other studies have shown that OPN is expressed in renal resident cells and is regarded as

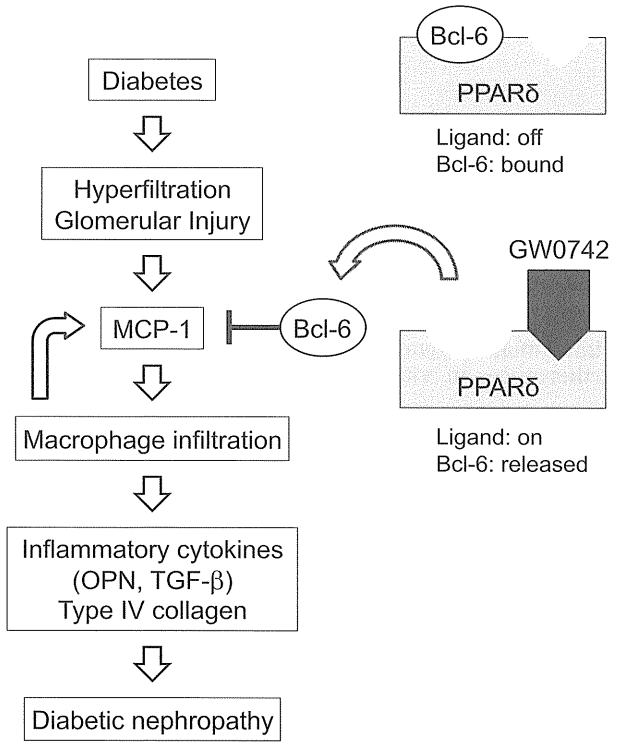

FIG. 7. Schematic diagram showing the mechanisms involved in the renoprotective effects of PPARδ in diabetic nephropathy. The anti-inflammatory transcriptional repressor Bcl-6 represses the expression of MCP-1. PPARδ activation by GW0742 releases Bcl-6, which is associated with suppression of MCP-1, to attenuate macrophage infiltration, inflammatory gene expression, and type IV collagen accumulation in the kidney.

a key molecule in the pathogenesis of diabetic nephropathy (20,21). In this study, consistent with the changes in MCP-1 expression, high glucose–induced OPN expression in macrophages was attenuated by GW0742. Therefore, inhibition of OPN expression by PPARδ agonists may contribute, at least in part, to the beneficial renal effects of PPARδ agonists in diabetic nephropathy.

In conclusion, the data presented in this study indicate that PPARδ activation attenuates high glucose–induced expression of MCP-1 and OPN by increasing the anti-inflammatory repressor Bcl-6 in macrophages. The PPARδ agonist GW0742 shows renoprotective effects through its anti-inflammatory activity by inhibiting MCP-1 expression and macrophage infiltration in the diabetic kidney. Because MCP-1 is a key component in the inflammatory response and the recruitment of monocytes/macrophages into the diabetic kidney, inhibition of MCP-1 expression by PPARδ agonist could provide a potential therapeutic target in human diabetic nephropathy.

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Y.M. conducted the research and contributed to discussion. D.O. wrote the manuscript, conducted research, and contributed to discussion. J.W. contributed to discussion and reviewed and edited the manuscript. N.Y. conducted research. K.S. contributed to discussion. C.S., H.T., and N.T. conducted research. H.M. contributed to discussion and reviewed and edited the manuscript.

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Original Article

Decreasing Abdominal Circumference Is Associated with Improving Estimated Glomerular Filtration Rate (eGFR) with Lifestyle Modification in Japanese Men: A Pilot Study

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The link between changes in a subject's metabolic syndrome components and his estimated glomerular filtration rate (eGFR) was evaluated in healthy Japanese men. We used data from 120 Japanese men (45.5 ± 8.4 years) with a 1-year follow up. eGFR was defined by a new equation developed for Japan. There were no significant differences in eGFR between men with and without metabolic syndrome components at baseline. Subjects were given advice for dietary and lifestyle improvement. At the 1-year follow up, almost all metabolic syndrome components were significantly improved. However, eGFR was significantly decreased. The changes in eGFR were weakly correlated with abdominal circumference ($r = -0.232$, $p = 0.0106$). A decrease in abdominal circumference may be associated with improving eGFR in Japanese men.

Key words: abdominal circumference, estimated glomerular filtration rate (eGFR), metabolic syndrome, lifestyle modification

Chronic kidney disease (CKD) has become a public health challenge and is a common disorder [1]. For example, approximately 20% of adults have CKD, which is defined as kidney damage or a glomerular filtration rate (GFR) $< 60\,ml/min/1.73\,m^2$ for at least 3 months, regardless of cause [2]. We have also previously reported in a cross-sectional study that the estimated glomerular filtration rate (eGFR) [3] in men with abdominal obesity and in women with hypertension was significantly lower than that in subjects without these components of metabolic syndrome [4]. In addition, we have shown that decreasing systolic blood pressure is associated with improving eGFR with lifestyle modification in healthy Japanese women [5]. However, whether decreases in metabolic syndrome components are beneficial for improving eGFR, and what effect this has on eGFR remain to be investigated in a longitudinal study in Japanese men.

In this study, we evaluated the link between changes in eGFR and changes in metabolic syndrome components in Japanese men with a 1-year follow up.

Subjects and Methods

Subjects. We used data for 120 Japanese men from a data-base of 16,383 people at the Okayama Southern Institute of Health in Okayama prefecture, Japan, aged 45.5 ± 8.4 years, who met the following criteria: (1) received a health check-up, including

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special health guidance and a follow-up check-up 1-year later; (2) received anthropometric measurements, fasting blood examination, and blood pressure measurements as part of the annual health check-up; (3) received no medications for diabetes, hypertension, and/or dyslipidemia; and (4) provided written informed consent (Table 1).

At the first health check-up, all subjects were given instructions by well-trained medical staff on how to change their lifestyle as special health guidance. Nutritional instruction was provided with a well-trained nutritionist, who planned a diet for each subject based on their data and provided simple instructions (*i.e.* not to eat too much and to consider balance when they eat). Exercise instruction was also provided by a well-trained physical therapist, who encouraged each subject to increase their daily number of steps walked.

Ethical approval for the study was obtained from the Ethical Committee of the Okayama Health Foundation.

Anthropometric and body composition measurements. Anthropometric and body compositions were evaluated based on the following parameters: height, body weight, and abdominal circumference. Body mass index (BMI) was calculated by weight / $[\text{height}]^2$, in kg/m^2. Abdominal circumference was measured at the umbilical level in standing subjects after normal expiration [6].

Blood pressure measurements at rest. Resting systolic and diastolic blood pressures were measured indirectly using a mercury sphygmomanometer placed on the right arm of the seated participant after at least 15 min of rest.

Urine examination. Urine samples were collected from the second-morning urine (before 10 a.m.) and subjected to examination within 1 h. The urine examination was performed using urine test strips (BAYER, Tokyo, Japan). The reagent strip was dipped directly into the urine sample. Just after dipping, the sample was graded as −: negative, ±: trace positive, +: positive (30 mg/dl), 2+: positive (100 mg/dl), 3+: positive (300 mg/dl), or 4+: positive (1,000 mg/dl) by comparison with a standard color chart found on the container's label.

Blood sampling and assays. We measured overnight fasting serum levels of creatinine (Cr) (enzymatic method), high-density lipoprotein (HDL) cholesterol, triglycerides (L Type Wako Triglyceride・H, Wako Chemical, Osaka, Japan), and blood sugar. eGFR was calculated using the following equation: $\text{eGFR}\ (\text{ml/min/1.73 m}^2) = 194 \times \text{Cr}^{-1.094} \times \text{Age}^{-0.287}$ [3]. Reduced eGFR was defined as an eGFR $< 60\ \text{ml/min/1.73 m}^2$.

Definition of metabolic syndrome. Men with an abdominal circumference in excess of 85 cm were defined as having metabolic syndrome if they also had two or more of the following components: 1) Dyslipidemia: triglycerides ≥ 150 mg/dl and/or HDL cholesterol < 40 mg/dl, 2) High blood pressure: blood pressure ≥ 130/85 mmHg, 3) Impaired glucose tolerance: fasting plasma glucose ≥ 110 mg/dl [6].

Table 1 Clinical characteristics and changes in parameters with 1-year follow up

	Baseline	Follow up	*p*
Number of Subjects	120		
Age	45.5 ± 8.4		
Height (cm)	169.0 ± 5.3		
Body weight (kg)	75.6 ± 11.3	74.0 ± 10.7	**<0.0001**
Body mass index (kg/m^2)	26.5 ± 3.6	25.9 ± 3.4	**<0.0001**
Abdominal circumference (cm)	88.5 ± 9.8	86.3 ± 9.2	**<0.0001**
Systolic blood pressre (mmHg)	131.5 ± 14.6	123.9 ± 12.5	**<0.0001**
Diastolic blood pressure (mmHg)	82.6 ± 11.5	77.0 ± 9.2	**<0.0001**
Triglyceride (mg/dl)	153.3 ± 110.2	121.7 ± 80.3	**0.0011**
HDL cholesterol (mg/dl)	54.2 ± 14.6	56.2 ± 14.9	**0.0390**
Blood sugar (mg/dl)	102.7 ± 18.1	104.2 ± 28.3	0.3710
Cr (mg/dl)	0.81 ± 0.12	0.84 ± 0.12	**0.0012**
eGFR ($ml/min/1.73 m^2$)	84.0 ± 13.9	80.1 ± 13.1	**<0.0001**
		Mean ± SD	

Statistical analysis. Data are expressed as means ± standard deviation (SD). A statistical analysis was performed using a paired t test and χ^2 test: $p < 0.05$ was considered to be statistically significant. Pearson's correlation coefficients were calculated and used to test the significance of the linear relationship among continuous variables.

Results

The clinical parameters at the baseline and the 1-year follow up are summarized in Table 1. Anthropometric and body composition parameters such as body weight, BMI, and abdominal circumference were significantly reduced with lifestyle modification after 1 year. Cr was significantly increased, and eGFR was decreased. Thirty-six subjects were diagnosed as having metabolic syndrome at baseline, and 18 subjects were diagnosed as having metabolic syndrome after 1 year, which was a significant reduction ($p < 0.0001$). Two subjects were diagnosed with reduced eGFR at baseline, and 3 subjects were diagnosed with reduced eGFR at the 1-year follow up. In addition, four subjects were identified as trace positive, 2 were identified as positive (+), and one was identified as positive (2+) for proteinuria at baseline, while 5 were identified as trace positive, 4 as positive (+), and 2 as positive (2+) at the 1-year follow up.

In subjects not taking medications, we also compared eGFR levels between the groups with and without each component of the Japanese definition of metabolic syndrome (Table 2). eGFR in men with abdominal obesity was significantly higher than that in men without abdominal obesity. However, there were no significant differences of eGFR between the groups with or without other components of metabolic syndrome. In addition, eGFR in subjects with metabolic syndrome was similar to that in subjects without it.

We further evaluated the relationship between changes in eGFR and changes in clinical parameters. Changes in eGFR were weakly correlated with changes in abdominal circumference ($r = -0.232$, $p = 0.0106$) (Table 3, Fig. 1). After we excluded one subject with abnormal changes in eGFR (-37.7 ml/min/1.73 m^2), changes in eGFR were still weakly correlated with changes in abdominal circumference ($n = 119$, $r = -0.203$, $p = 0.0265$). However, changes in eGFR were not significantly correlated with changes in other metabolic components.

Finally, we investigated the changes in eGFR amongst men with different levels of increased abdominal circumference [Group I: Delta (delta represents positive changes in abdominal circumference) abdominal circumference ≧ 0 cm, Group D: Delta abdominal circumference < 0 cm]. After the 1-year follow up, changes in eGFR in Group I (-6.0 ± 10.2ml/min/1.73 m^2) were lower than those in Group D (-2.7 ± 8.2ml/min/1.73 m^2), but not at a significant level ($p = 0.0599$).

Table 2 Comparison of eGFR between men with and without metabolic syndrome

	Abdominal obesity (−)	Abdominal obesity (+)	p
Number of subjects	42	78	
eGFR (ml/min/1.73 m^2)	79.1 ± 13.9	86.6 ± 13.2	**0.0042**
	Impaired glucose torelance (−)	Impaired glucose torelance (+)	
Number of subjects	96	24	
eGFR (ml/min/1.73 m^2)	82.7 ± 13.7	88.8 ± 13.8	0.0535
	Hypertension (−)	Hypertension (+)	
Number of subjects	48	72	
eGFR (ml/min/1.73 m^2)	83.2 ± 14.8	84.5 ± 13.3	0.6326
	Dyslipidemia (−)	Dyslipidemia (+)	
Number of subjects	71	49	
eGFR (ml/min/1.73 m^2)	82.3 ± 14.1	86.2 ± 13.5	0.1348
	Metabolic syndrome (−)	Metabolic syndrome (+)	
Number of subjects	84	36	
eGFR (ml/min/1.73 m^2)	82.4 ± 14.0	87.5 ± 13.2	0.0644

Mean ± SD

Table 3 Simple correlation analysis between changes in eGFR andchanges in clinical parameters with 1-year follow up

	r	p
Abdominal circumference (cm)	−0.232	**0.0106**
Systolic blood pressre (mmHg)	0.094	0.3068
Diastolic blood pressure (mmHg)	−0.009	0.9227
Triglyceride (mg/dl)	−0.055	0.5521
HDL cholesterol (mg/dl)	−0.016	0.8616
Blood sugar (mg/dl)	−0.030	0.7458

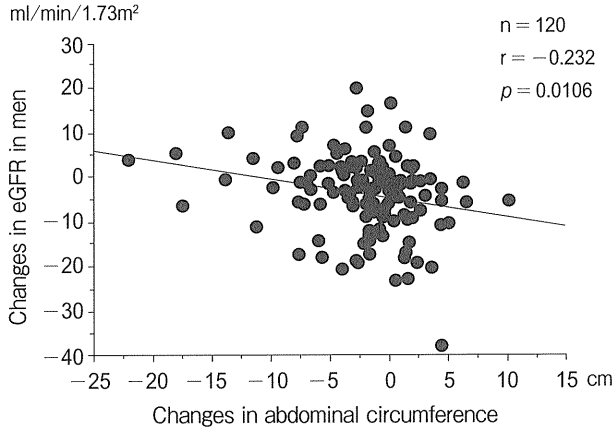

Fig. 1 Simple correlation analysis between changes in eGFR and changes in systolic blood pressure at the 1-year follow up.

Discussion

The main objective of this study was to explore the link between changes in eGFR and changes in metabolic syndrome components in Japanese men with a 1-year follow up.

Ninomiya T *et al.* [7], Tanaka *et al.* [8] and Iseki *et al.* [9] reported that metabolic syndrome, using the modified ATP III definition [10], was associated with CKD in the Japanese population. Compared with subjects with 0 or 1 components of metabolic syndrome, subjects with 2, 3, and 4 or more components had odds ratios of 1.13, 1.90, and 2.79 for CKD [7]. In this study, 36 subjects was diagnosed as having metabolic syndrome, using the Japanese criteria, at baseline, and 18 were diagnosed as having metabolic syndrome at the 1-year follow up. We have previously reported a prevalence of 30.7% for metabolic syndrome in Japanese men [11]. In this study, with lifestyle modification after the initial health check-up, metabolic components were significantly improved in men without medications at the 1-year follow-up. Although eGFR was not increased after 1 year, changes in eGFR were negatively correlated with changes in abdominal circumference. Taken together, lifestyle modification targeting reducing abdominal circumference may be a useful method for improving eGFR in Japanese men.

Abdominal obesity contributes to the development of renal injury and end-stage renal disease [12–14]. Bonnet *et al.* have reported that abdominal obesity is related to the development of elevated albuminuria in both sexes, suggesting that the measurement of abdominal circumference might improve the identification of non-diabetic individuals at risk of developing microalbuminuria [12]. In addition, a greater waist-to-hip ratio is associated with a greater risk of diminished filtration, even when corrected for BMI [13]. Yamagata *et al.* have reported that the baseline-adjusted predictor of developing CKD included age, GFR, hematuria, hypertension, diabetes, serum lipids, obesity, smoking status, and consumption of alcohol with a 10-year follow up [14]. In the present study, there were significant differences in eGFR between subjects with and without abdominal obesity at baseline. However, we revealed that, with lifestyle modification, changes in abdominal circumference were weakly correlated with changes in eGFR in men without medications. Changes in other metabolic components were not linked to changes in eGFR. Therefore, the clinical impact of adnominal circumference on eGFR was noted in Japanese men.

Potential limitations remain in our study. First, the 16,383 subjects in our study voluntarily underwent the annual health check-up; they were, therefore, probably more health-conscious than the average person. The selected 120 men underwent an annual health check-up every year with a follow-up duration of 1-year and received no medication; they were, therefore, probably even more health-conscious than most of the subjects in the database, and the small sample size may make it difficult to infer causality between eGFR and abdominal circumference. At baseline, in contrast to our previous report regarding a large sample (n = 11,711) from a cross-sectional study [4], eGFR in men with abdominal obesity was higher than that in men without abdominal obesity. eGFR was not increased with lifestyle modification after 1 year

($-3.9\,ml/min/1.73\,m^2$/year). A link has previously been found between eGFR and age, based on a large sample from a Japanese cohort, with an average decline rate of eGFR of $0.36\,ml/min/1.73\,m^2$/year [15]. Therefore, the decline rate of eGFR in our study was higher than that previously reported. Second, we could not identify the mechanism of the linkage between eGFR and abdominal circumference. Third, most of the enrolled subjects were not diagnosed as CKD at baseline. Therefore, the results in this study may not apply to patients with CKD. Further prospective studies are needed in Japanese subjects.

In conclusion, a decrease in abdominal circumference with lifestyle modification might induce an improvement in eGFR. Therefore, lifestyle modification may be a necessary and useful measure for the prevention of CKD in Japanese men.

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SHORT COMMUNICATION

The relation between estimated glomerular filtration rate and proteinuria in Okayama Prefecture, Japan

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Abstract
Objective We investigated the link between renal function as evaluated by estimated glomerular filtration rate (eGFR) and proteinuria in Okayama Prefecture, Japan.
Subjects and methods A total of 11030 Japanese subjects, aged between 20 and 79 years, were recruited in a cross-sectional clinical investigation study. eGFR was calculated using serum creatinine, age, and sex. Proteinuria was measured by using urine strip devices.
Results Age-related variations in eGFR were noted. Two hundred sixteen men (6.2%) and 316 women (4.2%) were diagnosed with trace positive (±) and 140 men (4.0%) and 130 women (1.7%) were diagnosed with positive (+≤) proteinuria. eGFR in subjects with +≤ proteinuria was significantly lower than that in subjects without proteinuria, in both sexes.
Conclusion The present study indicates that proteinuria might be an important marker in the etiology of lower eGFR in Okayama Prefecture, Japan.

Keywords Estimated glomerular filtration rate (eGFR) · Proteinuria · Prevalence

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Introduction

Chronic kidney disease (CKD) has become an important public health challenge in Japan, and it is a major risk factor for end-stage renal disease, cardiovascular disease, and premature death [1, 2]. Identifying and taking care of risk factors for early CKD may be the best approach to prevent and delay adverse outcomes from happening prematurely [1]. The Japanese Society of Nephrology recently established an equation for estimating glomerular filtration rate (GFR) from serum creatinine (Cr) and age for the Japanese general population [3]. The new equation provides reasonably accurate estimated GFR (eGFR) values for clinical practice and epidemiological study. Imai et al. [4] reported that approximately 13.3 million people were predicted to have CKD in 2005.

We have previously shown that eGFR in men with abdominal obesity and in women with hypertension was significantly lower than in those without such conditions in a cross-sectional study [5]. In addition, decreased systolic blood pressure was closely linked to improved eGFR in 53 Japanese healthy women in a 1-year follow-up study using the new equation for the Japanese [6].

It is well known that proteinuria promotes renal dysfunction. However, the link between proteinuria and eGFR calculated with the new equation remains to be investigated. Therefore, we evaluated eGFR and its relation to

prevalence of proteinuria in a large sample of Japanese population in Okayama Prefecture, Japan.

Subjects and methods

Subjects

We used data of 11030 Japanese subjects from a database of 16383 people at Okayama Southern Institute of Health in Okayama Prefecture, Japan, aged between 20 and 79 years in a cross-sectional study (Table 1). All subjects met the following criteria: (1) he or she had received an annual health checkup from June 1999 to May 2008 at Okayama Southern Institute of Health; (2) he or she had received Cr, urine examination, and anthropometric measurements as part of their annual health checkups; and (3) he or she provided us with written informed consent. Ethical approval for the study was obtained from the Ethical Committee of Okayama Health Foundation (2010-9).

Anthropometric measurements

Anthropometric parameters were evaluated by using the respective parameters such as height, body weight, body mass index (BMI), and abdominal circumference. BMI was calculated as weight/[height]^2 (kg/m^2). Abdominal circumference was measured at the umbilical level of a standing subject after normal expiration [7].

Blood sampling and assays

We measured overnight-fasting serum levels of Cr [3] (enzymatic method). eGFR was calculated using the following equation: eGFR (ml/min/1.73 m^2) = 194 $\times$ Cr$^{-1.094}$ $\times$ Age$^{-0.287}$ $\times$ 0.739 (if woman) [3]. Reduced eGFR was defined as eGFR <60 ml/min/1.73 m^2.

Urine examination

Urine samples were collected from the second morning urine (before 10 a.m.) and examined within 1 h. Urine examination was performed using urine strip tests (Bayer, Tokyo, Japan). The reagent strip was dipped directly into the urine sample. Just after dipping, the sample was graded as: −, negative; ±, trace positive; +, positive (30 mg/dl); 2+, positive (100 mg/dl); 3+, positive (300 mg/dl); or 4+, positive (1000 mg/dl) by comparison with a standard color chart found on the container's label [8].

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Comparison of parameters was performed by one-way analysis of variance (ANOVA), Scheffe's F test, and logistic regression analysis, with $p < 0.05$ considered statistically significant. Statistical analysis was performed using StatView 5.0 (SAS Institute Inc., Cary, NC, USA).

Results

The clinical profile of the subjects is summarized in Table 1. eGFR was 85.3 ± 18.6 ml/min/1.73 m^2 in men and 91.2 ± 22.6 ml/min/1.73 m^2 in women. eGFR classified by age group is summarized in Table 2. eGFR decreased significantly with age in subjects over 30 years old, and eGFR in the eighth decade was similar to that in the seventh decade for both sexes. A total of 253 men (7.3%) and 417 women (5.5%) were diagnosed with reduced eGFR. Prevalence of subjects with reduced eGFR increased with age.

We evaluated prevalence of proteinuria; 216 men (6.2%) and 316 women (4.2%) were diagnosed with ±, while 140 men (4.0%) and 130 women (1.7%) were diagnosed with +≤ proteinuria (Table 2). Prevalence of

Table 1 Clinical profile of the Japanese subjects

	Men (n = 3467)			Women (n = 7563)		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
Age (years)	43.1 ± 13.9	20	79	42.4 ± 14.0	20	79
Height (cm)	169.0 ± 6.2	143.7	187.6	156.3 ± 5.7	134.9	179.3
Body weight (kg)	70.2 ± 11.5	39.1	142.4	55.1 ± 9.0	32.1	116.9
BMI (kg/m^2)	24.6 ± 3.6	13.6	45.4	22.6 ± 3.6	12.9	48.7
Abdominal circumference (cm)	84.2 ± 10.1	58.0	132.1	72.1 ± 9.6	43.3	123.6
Cr (mg/dl)	0.83 ± 0.15	0.39	1.90	0.60 ± 0.16	0.20	6.8
eGFR (ml/min/1.73 m^2)	85.3 ± 18.6	27.8	191.3	91.2 ± 22.6	7.0	260.0

BMI body mass index, *Cr* creatinine, *eGFR* estimated glomerular filtration rate

Table 2 Changes in eGFR and proteinuria, classified by age group

Age (years)	Number of subjects	eGFR Mean ± SD	Number of subjects with reduced eGFR	%	Proteinuria (−)	%	(±)	%	(+≤)	%
Men										
20–29	714	99.8 ± 16.9	0	0.0	648	90.7	47	6.6	19	2.7
30–39	850	89.8 ± 16.3[a]	11	1.3	766	90.1	54	6.5	30	3.5
40–49	724	81.9 ± 15.3[a,b]	45	6.2	645	89.1	54	7.5	25	3.5
50–59	633	77.9 ± 16.1[a,b,c]	63	10.0	570	90.0	31	4.9	32	5.1
60–69	441	73.1 ± 15.8[a,b,c,d]	102	23.1	393	89.1	25	5.7	23	5.2
70–79	105	68.1 ± 14.5[a,b,c,d]	32	30.5	89	84.7	5	4.8	11	10.5
Total	3467	85.3 ± 18.6	253	7.3	3111	89.7	216	6.2	140	4.0
Women										
20–29	1832	106.7 ± 21.9	10	0.5	1697	92.6	101	5.5	34	1.9
30–39	1703	96.8 ± 20.3[a]	29	1.7	1608	94.4	70	4.1	25	1.5
40–49	1483	87.6 ± 19.0[a,b]	66	4.5	1396	94.1	60	4.0	27	1.8
50–59	1459	81.2 ± 18.7[a,b,c]	141	9.7	1386	95.0	51	3.5	22	1.5
60–69	921	75.5 ± 16.4[a,b,cd]	133	14.4	875	95.0	28	3.0	18	2.0
70–79	165	70.8 ± 16.3[a,b,c,d]	38	23.0	155	93.9	6	3.6	4	2.4
Total	7563	91.2 ± 22.6	417	5.5	7117	94.1	316	4.2	130	1.7

eGFR estimated glomerular filtration rate

[a] $p < 0.05$ versus age 20–29 years

[b] $p < 0.05$ versus age 30–39 years

[c] $p < 0.05$ versus age 40–49 years

[d] $p < 0.05$ versus age 50–59 years

+≤ proteinuria was highest in the eighth decade for both sexes.

We evaluated the relationship between eGFR and proteinuria (Table 3). Prevalence of proteinuria was closely linked to reduced eGFR. eGFR in subjects with +≤ proteinuria was significantly lower than that in subjects without proteinuria, for both sexes. Twenty-one men (15.0%) and 21 women (16.2%) in subjects with +≤ proteinuria were diagnosed as having reduced eGFR (Table 3).

We also compared the relationship between eGFR and proteinuria as classified by age group (Table 3). eGFR in women with ± proteinuria in their third and fifth decades was significantly lower than that in women without proteinuria. In addition, eGFR in women with +≤ proteinuria in their third and seventh decades was also significantly lower than that in women without proteinuria. In other age groups, eGFR in subjects with proteinuria was also lower than that in subjects without proteinuria, but not significantly so. On logistic regression analysis, there was significant difference in eGFR after adjusting for age in women ($p < 0.0001$). However, significant difference of eGFR was not noted after adjusting for age in men ($p = 0.0960$).

Discussion

In this study, we explored eGFR and its relation to proteinuria. eGFR was closely linked to proteinuria, especially in women of Okayama Prefecture in Japan.

It is well known that prevalence of proteinuria increases with age, and its rate among Japanese is reported to be 3.2% by Imai et al. [4]. eGFR also decreases with age [9]. Regarding the link between eGFR and age, in the large sample of another Japanese cohort, the rate of decrease of eGFR was 0.36 ml/min/1.73 m^2/year [9]. In this study, we also found that prevalence of proteinuria was highest in the eighth decade, and eGFR decreased with age on cross-sectional analysis.

Several studies have documented the relationship between proteinuria and end-stage renal disease [9, 10]. Imai et al. reported that the rate of decrease of eGFR, using the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation modified by a Japanese coefficient, was more than two times higher in participants with proteinuria than in those without it [9]. Iseki et al. [10] also reported that they identified a strong, graded relationship between end-stage renal disease and positive dipstick urinalysis for proteinuria (adjusted odds ratio 2.71).

Table 3 Relationship between eGFR and proteinuria, classified by age group

Age (years)	Proteinuria		
	(−)	(±)	(+≤)
Men			
20–29	100.0 ± 16.9	98.3 ± 18.2	94.9 ± 15.6
30–39	90.4 ± 16.1	85.3 ± 17.5	84.4 ± 16.0
40–49	82.0 ± 15.2	81.1 ± 14.5	81.0 ± 18.9
50–59	77.7 ± 15.5	84.3 ± 19.7	76.9 ± 20.9
60–69	73.0 ± 15.1	75.9 ± 24.1	70.9 ± 16.3
70–79	69.1 ± 12.8	63.7 ± 24.7	61.2 ± 21.0
Total	85.5 ± 18.4	85.3 ± 19.8	79.5 ± 19.9[a,b]
Number of subjects with reduced eGFR (%)	216 (6.9)	16 (7.4)	21 (15.0)
Women			
20–29	107.3 ± 21.9	101.0 ± 20.9[a]	94.1 ± 19.9[a]
30–39	96.8 ± 20.2	98.1 ± 20.6	92.9 ± 21.2
40–49	88.0 ± 19.1	80.4 ± 15.6[a]	85.6 ± 16.2
50–59	81.3 ± 18.7	77.9 ± 19.4	76.8 ± 18.6
60–69	75.8 ± 15.9	72.2 ± 22.2	62.5 ± 25.2[a]
70–79	71.2 ± 16.5	64.8 ± 11.8	62.3 ± 8.3
Total	91.4 ± 22.6	89.5 ± 22.7	83.8 ± 22.6[a]
Number of subjects with reduced eGFR (%)	374 (5.3)	22 (7.0)	21 (16.2)

Mean ± SD

[a] $p < 0.05$ versus (−)

[b] $p < 0.05$ versus (±)

In addition, macroalbuminuria was a better risk marker than low eGFR or erythrocyturia to identify individuals at risk for accelerated GFR loss in population screening with 4-year follow-up [11]. Therefore, proteinuria is a strong, independent predictor of end-stage renal disease. Our study also showed that eGFR in subjects with +≤ proteinuria was significantly lower than that in subjects without proteinuria. Prevalence of subjects with reduced eGFR among subjects with proteinuria was also higher than that in subjects without it. About 15% were diagnosed with reduced eGFR among subjects with +≤ proteinuria. However, the significant relationships between eGFR and proteinuria were attenuated by separate analysis by age group classification and logistic regression analysis. The small sample size of subjects with proteinuria may have affected these results.

Potential limitations still remain in our study. First of all, the cross-sectional study design we used makes it difficult to infer association between proteinuria and eGFR. Secondly, we are yet to prove directly the mechanism of the link between proteinuria and eGFR. Thirdly, although we had reported the clinical impact of some factors on eGFR [5, 6], we are yet to evaluate these factors, including hemoglobin A1c. However, our findings are applicable to clinical and public health practice settings. In conclusion, prevalence of proteinuria is associated with lower eGFR, especially in women, in Okayama Prefecture, Japan. Further prospective studies are necessary to investigate the link between eGFR and proteinuria in the Japanese population in general.

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