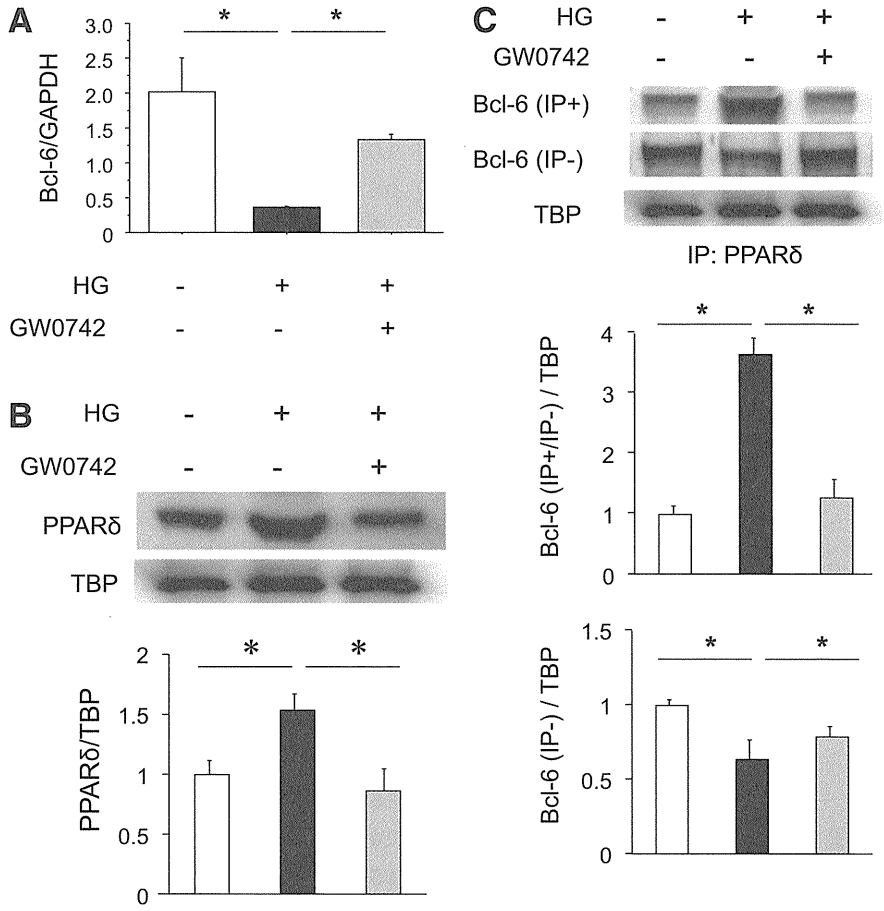

FIG. 5. High glucose suppresses Bcl-6 expression in RAW macrophages. *A*: mRNA isolated from macrophages was analyzed by quantitative RT-PCR. Bcl-6 expression was normalized to GAPDH. Data are means ± SE. **P* < 0.05. *B*: High glucose increases and GW0742 decreases macrophage PPARδ protein expression. 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

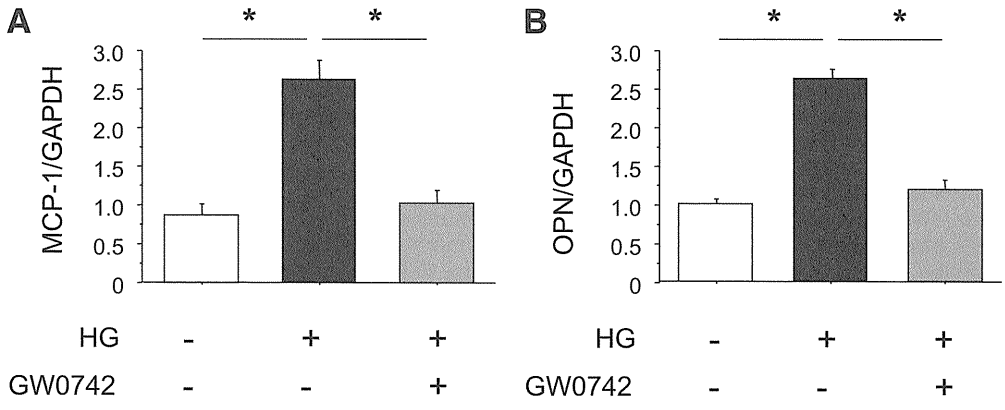

FIG. 6. GW0742 suppresses MCP-1 (*A*) and OPN (*B*) mRNA expression in RAW macrophages. mRNA isolated from macrophages was analyzed by quantitative RT-PCR. MCP-1 and OPN expression was normalized to GAPDH. Data are means ± SE. **P* < 0.05.

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

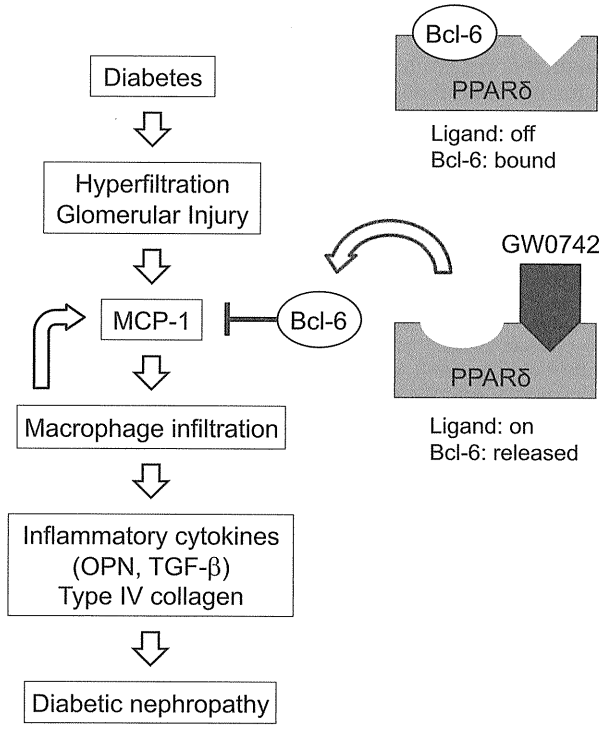

FIG. 7. Schematic diagram showing the mechanisms involved in the renoprotective effects of PPARδ in diabetic nephropathy. The anti-inflammatory transcriptional repressor Bcl-6 represses the expression of MCP-1. PPARδ activation by GW0742 releases Bcl-6, which is associated with suppression of MCP-1, 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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Research Article

High Glucose Increases Metallothionein Expression in Renal Proximal Tubular Epithelial Cells

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Metallothionein (MT) is an intracellular metal-binding, cysteine-rich protein, and is a potent antioxidant that protects cells and tissues from oxidative stress. Although the major isoforms MT-1 and -2 (MT-1/-2) are highly inducible in many tissues, the distribution and role of MT-1/-2 in diabetic nephropathy are poorly understood. In this study, diabetes was induced in adult male rats by streptozotocin, and renal tissues were stained with antibodies for MT-1/-2. MT-1/-2 expression was also evaluated in mProx24 cells, a mouse renal proximal tubular epithelial cell line, stimulated with high glucose medium and pretreated with the antioxidant vitamin E. MT-1/-2 expression was gradually and dramatically increased, mainly in the proximal tubular epithelial cells and to a lesser extent in the podocytes in diabetic rats, but was hardly observed in control rats. MT-1/-2 expression was also increased by high glucose stimulation in mProx24 cells. Because the induction of MT was suppressed by pretreatment with vitamin E, the expression of MT-1/-2 is induced, at least in part, by high glucose-induced oxidative stress. These observations suggest that MT-1/-2 is induced in renal proximal tubular epithelial cells as an antioxidant to protect the kidney from oxidative stress, and may offer a novel therapeutic target against diabetic nephropathy.

1. Introduction

Diabetic nephropathy is a leading cause of end-stage renal disease, and many mechanisms have been proposed to explain the pathogenesis of renal injury in diabetes [1]. Recent studies have shown that hyperglycemia may induce oxidative stress by increasing reactive oxygen species (ROS) generation in the diabetic kidney [2–4] and that overexpression of the antioxidant superoxide dismutase 1 attenuated diabetic nephropathy in streptozotocin (STZ)-induced and *db*/*db* diabetic mice [5, 6]. Therefore, ROS could be an important mediator of diabetic nephropathy, and protection from ROS might offer a valuable therapeutic strategy to treat diabetic nephropathy.

Metallothionein (MT) is an intracellular metal-binding protein with a low-molecular mass (6-7 kDa) and a high cysteine content (20 of 61-62 amino acids). Its major isoforms, MT-1 and -2 (MT-1/-2), are widely distributed

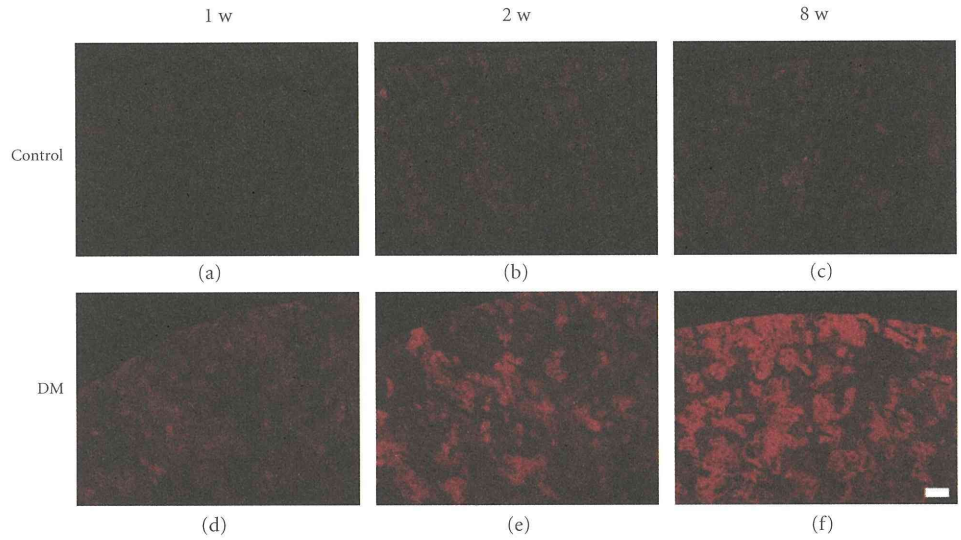

FIGURE 1: MT-1/-2 expression in the kidney. Diabetes was induced by injection of streptozotocin, and kidneys were obtained at 1 (a and d), 2 (b and e), or 8 (c and f) weeks after inducing diabetes. Immunofluorescent staining was performed as described in *Materials and Methods*. MT was strongly expressed in the renal cortex of diabetic rats (d, e, f) and hardly expressed in control rats (a, b, c). The expression of MT-1/-2 was greater at week 8 than at weeks 1 and 2 after diabetes induction. Scale bar: 100 μm.

throughout the body [7, 8]. Since MT-1/-2 expression is significantly upregulated by overload of essential trace metals (e.g., Zn and Cu), it plays an important role in heavy metal detoxification and essential metal homeostasis [9, 10]. In addition, MTs have been shown to act as nonspecific free radical scavengers [11, 12], suggesting that they exert antioxidant activities in various diseases, including diabetic nephropathy.

We and other investigators have demonstrated that MTs have neuroprotective effects in mouse models of Parkinson's disease [13–15]. In contrast, the role of MTs in the pathogenesis of diabetic nephropathy is poorly understood. Several studies reported that renal expression of MT is increased in STZ-induced diabetic rats [16], diabetic BB rats [17], and *ob/ob* diabetic mice [18]. However, the distribution of MTs in the diabetic kidney and the mechanisms by which MTs are induced in diabetes are poorly understood. Therefore, in the present study, we investigated the expression and localization of MT-1/-2 during the development of diabetic nephropathy and explored the mechanism by which MT-1/-2 expression was induced by high glucose in the kidney.

2. Materials and Methods

2.1. Experimental Protocol. Male Sprague Dawley rats were purchased from Charles River (Yokohama, Japan). Five-week-old rats were divided into two groups: (1) nondiabetic control rats (control; $n = 6$) and (2) STZ-induced diabetic rats (DM; $n = 6$). Diabetes was induced by peritoneal injection of 200 mg/kg STZ (Sigma-Aldrich Corp., MO) in citrate buffer (pH 4.5). Blood glucose was measured by the glucose oxidase method at 3 days after STZ injection and only rats with blood glucose concentrations >16 mmol/L were used in the study. All rats 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 the Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). Rats were sacrificed at 1, 2, or 8 weeks after inducing diabetes. We measured body weight, hemoglobin A1c (HbA1c), and 24-h urinary albumin excretion (UAE) at 1, 2, and 8 weeks. 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.

2.2. Immunofluorescent Staining of MT-1/-2 in Rat Kidney. Immunofluorescent staining was performed as previously described [19]. Renal expression of MT-1/-2 was detected using mouse anti-MT-1/-2 antibody (Dako, Carpinteria, CA) followed by Alexa Fluor 594 goat anti-mouse IgG (Invitrogen, Carlsbad, CA). To determine whether MT-1/-2 was localized in podocytes or proximal tubular epithelial cells, the sections were counterstained with guinea pig antinephrin antibody (Fitzgerald, Concord, MA) or rabbit antiaquaporin 1 antibody (Millipore, Billerica, MA), followed by Alexa Fluor 488 goat anti-guinea pig IgG or anti-rabbit IgG (Invitrogen), respectively. Fluorescence images were obtained using a fluorescence microscope (BX51; Olympus, Tokyo, Japan).

2.3. Cell Culture and Treatment. mProx24 cells, a murine renal proximal tubular epithelial cell line derived from

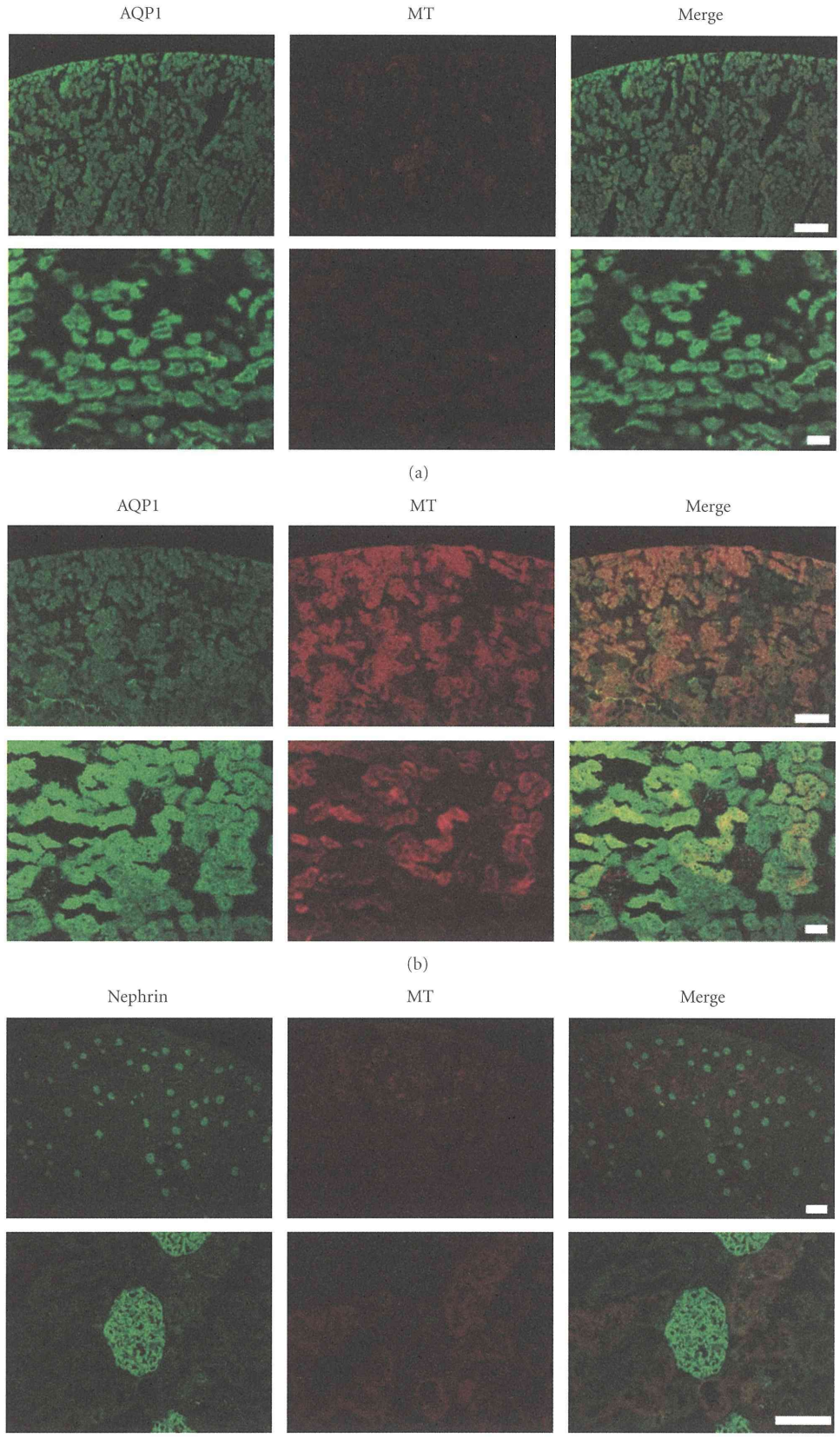

FIGURE 2: Continued.

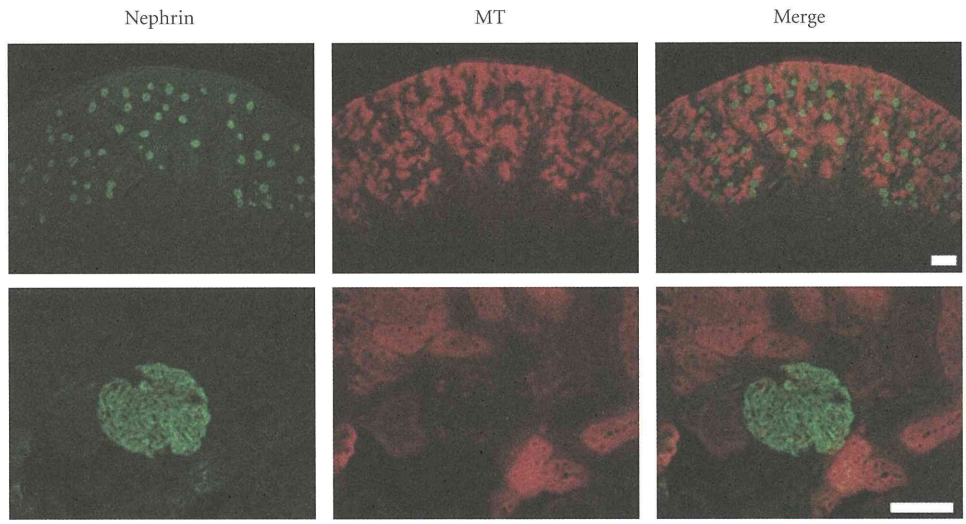

FIGURE 2: MT-1/-2 expression in podocytes and proximal tubular cells of the kidney. Immunofluorescent staining was performed as described in *Materials and Methods*. Eight weeks after inducing diabetes, MT-1/-2 was predominantly expressed in the proximal tubular epithelial cells of the kidney (b) and weakly expressed in podocytes (d) in the kidney of diabetic rats. In control rats, MT-1/-2 was weakly expressed in proximal tubular epithelial cells (a), but hardly in the podocytes (c). AQP1: aquaporin 1, MT: MT-1/-2. Scale bar: upper panels, 200 μm; lower panels, 50 μm.

C57BL/6J adult mouse kidney [20], were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich Corp.) supplemented with 1000 mg/L D-glucose, 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in 5% CO_2. To evaluate the effect of high glucose on MT expression, the cells were serum-starved by culture in 0.5% FBS for 24 h, then stimulated with 4500 mg/L D-glucose (high glucose) or D-mannitol (Sigma-Aldrich Corp.) for 24 h. For antioxidant treatment, the cells were pretreated with vitamin E (Sigma-Aldrich Corp.) at concentration ranges from 20 to 200 nM for 24 h, then stimulated with high glucose for 24 h. Individual experiments were repeated at least three times with different lots or preparations of cells.

2.4. Quantitative Analyses of MT-1 Gene and MT-1/-2 Protein Expression in mProx Cells. RNA was isolated from mProx cells 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 MT-1 in mProx24 cells, quantitative RT-PCR (qRT-PCR) was performed using StepOnePlus (Applied Biosystems, Tokyo, Japan) and Fast-Start SYBR Premix Ex Taq II (Takara Bio Inc., Otsu, Japan). The primers for the MT-1 gene (upstream 5′-TCTAAGCGTCACCACGACTTCA-3′ and downstream 5′-GTGCACTTGCAGTTCTTGCAG-3′) were purchased from Takara Bio Inc. Each sample was analyzed in triplicate and normalized for GAPDH mRNA expression. Immunofluorescent staining of MT-1/-2 protein was performed as described above. The immunofluorescence intensity in cultured mProx cells was calculated using the formula, x (density) × positive area (μm^2), using Lumina Vision software (Mitani Corporation).

2.5. Statistical Analysis. All values are means ± SEM. Statistically significant differences between groups were examined using one-way ANOVA followed by Scheffé's test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. MT-1/-2 Expression Was Increased in Diabetic Kidney. MT-1/-2 expression was observed in the renal cortex from 1 week after the induction of diabetes. Its expression increased gradually and was strongly upregulated at week 8 (Figure 1,(d),(e),(f)). In contrast, MT-1/-2 was hardly detected in the kidney of control rats (Figure 1, (a),(b),(c)). Renal sections counterstained with antiaquaporin 1 and antinephrin antibodies revealed that MT-1/-2 expression was predominantly localized in the proximal tubular epithelial cells (Figure 2(b)), and to a lesser extent in the podocytes of the diabetic kidneys (Figure 2(d)). In control rats, MT-1/-2 was weakly expressed in the proximal tubular epithelial cells (Figure 2(a)), but not in the podocytes (Figure 2(c)). Body weight, kidney weight, UAE, and HbA1c are shown in Table 1. Diabetic rats had a significantly lower body weight and higher kidney weight per body weight at 8 weeks, but not at 1 and 2 weeks after the induction of diabetes. Similarly, The UAE and HbA1c level in the diabetic rats was significantly higher than in the control rats at 8 weeks, but not at 1 and 2 weeks. Glomerular hypertrophy and mesangial matrix expansion, but not interstitial changes and tubular atrophy

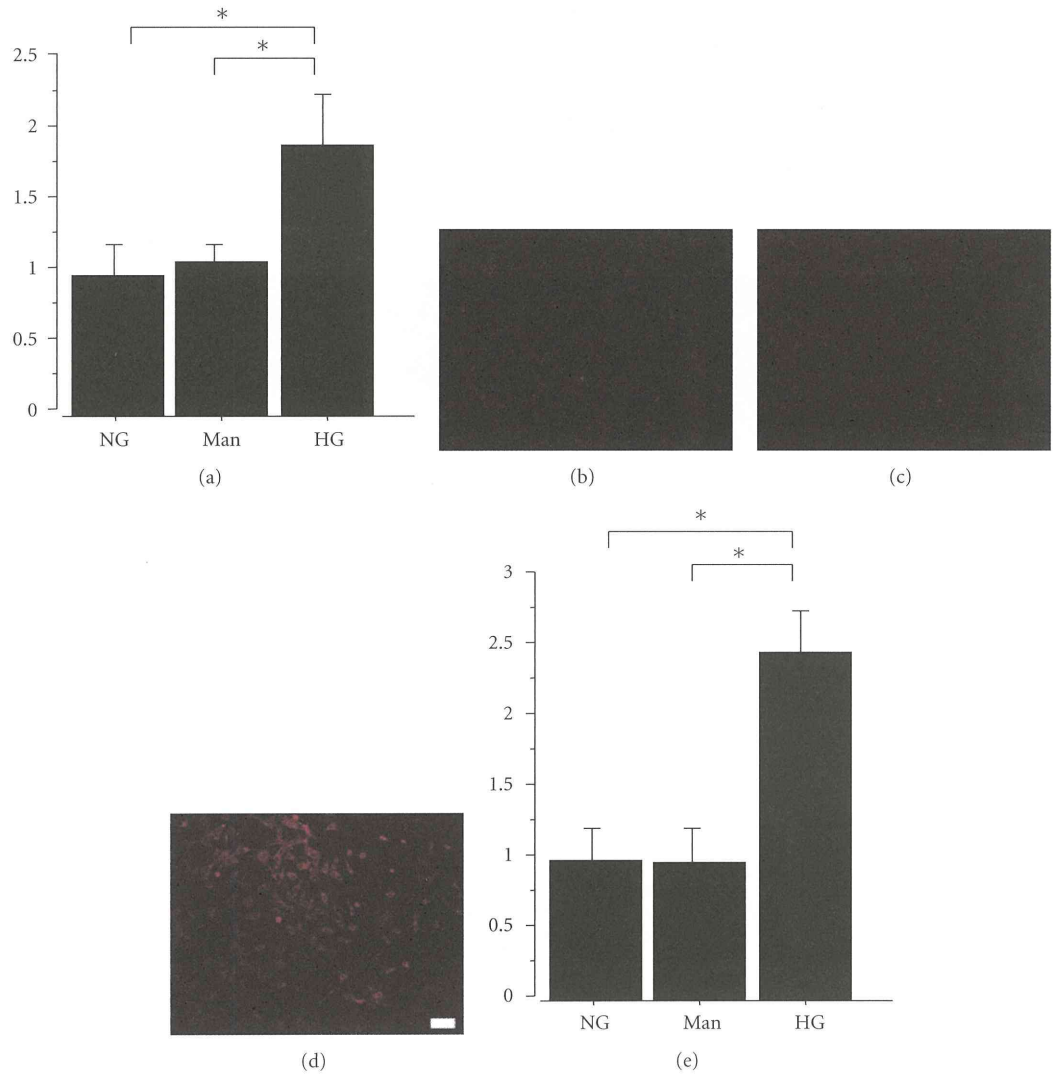

FIGURE 3: High glucose increases MT-1 mRNA and MT-1/-2 protein expression. mProx24 cells were serum-starved for 24 h before stimulation with high glucose or mannitol. (a) Cells were harvested after 24 h, and MT-1 mRNA expression was analyzed by qRT-PCR in three independent experiments and normalized for GAPDH. (b–e) MT-1/-2 protein expression was determined by immunofluorescent staining with anti-MT-1/-2 antibody 24 h after stimulation followed by densitometric analysis. Results are means ± SEM of three independent experiments. $^*P < 0.05$ versus high glucose; NG: normal glucose; Man: mannitol; HG: high glucose. Scale bar: 100 μm.

were observed in the diabetic rats as compared with control rats at 8 weeks (data not shown).

3.2. High Glucose Increased MT-1/-2 Expression in mProx24 Cells. qRT-PCR analyses revealed that exposure to the high glucose medium significantly increased MT-1 mRNA expression in mProx24 cells compared with normal glucose medium (Figure 3(a)). Similarly, high glucose, but not mannitol, significantly increased MT-1/-2 protein expression in mProx24 cells (Figures 3(b)–3(e)). These data indicate that high glucose increases the mRNA and protein expression of MT-1/-2 in mProx24 cells.

3.3. MT-1/-2 Expression Was Suppressed by Vitamin E. It is well known that high glucose increases the generation of ROS in various cells. To investigate the mechanism by which MT is induced by ROS in the high glucose condition, we examined the effects of an antioxidant, vitamin E, on MT-1/-2 expression in mProx24 cells. As shown in Figure 4, high-glucose-stimulated MT-1/-2 expression was significantly attenuated by vitamin E in a dose-dependent manner (Figure 4). Accordingly, these findings suggest that ROS generated by high glucose induces MT-1/-2 expression in the proximal tubular epithelial cells of the kidney.

4. Discussion

There is increasing evidence from experimental and clinical studies to suggest that oxidative stress plays a critical role in the pathogenesis and progression of diabetic

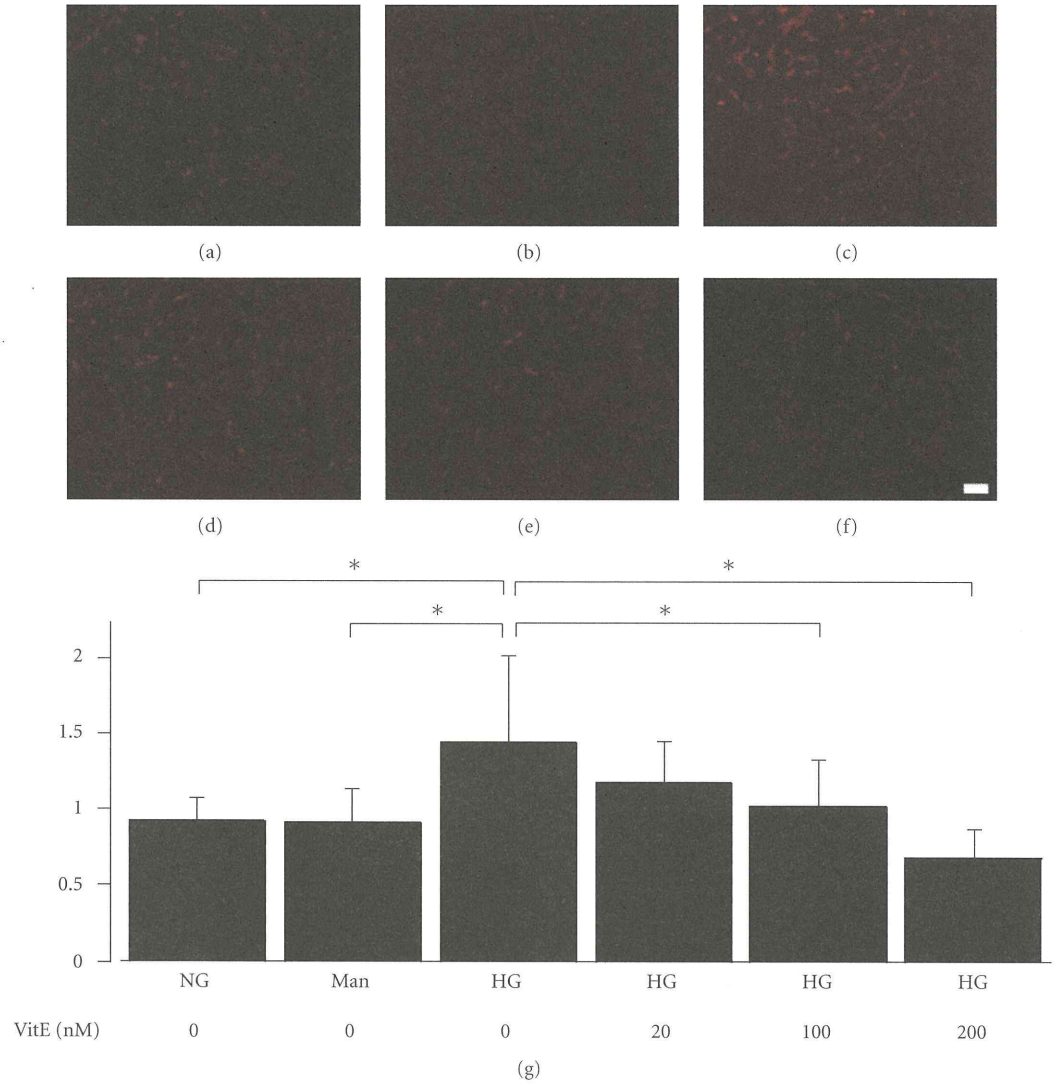

FIGURE 4: Vitamin E suppresses high glucose-induced MT-1/-2 expression. mProx24 cells were serum-starved and pretreated with vehicle or vitamin E for 24 h before stimulation with high glucose or mannitol. MT-1/-2 expression was determined by immunofluorescent staining. MT-1/-2 expression was not increased by mannitol (b) compared with normal glucose (a), but was increased by high glucose (c). High glucose-induced MT-1/-2 expression was attenuated by vitamin E pretreatment in a dose-dependent manner (d: 20 nM; E: 100 nM; F: 200 nM). The cells depicted are representative of three independent experiments. (g) Densitometric quantification of MT-1/-2 immunofluorescence. Results are means ± SEM of three independent experiments. $^*P < 0.05$ versus high glucose; NG: normal glucose; Man: mannitol; HG: high glucose; Vit E: vitamin E. Scale bar: 100 μm.

complications [21]. Since MT is a potent, endogenous and inducible antioxidant in various tissues [11, 12], we hypothesized that MT may be induced and act as an antioxidant in STZ-induced diabetic kidneys. Here, we found that high glucose induces the expression of MT-1/-2 mainly in proximal tubular epithelial cells and, to a lesser extent, in podocytes in rat kidneys. MT-1/-2 was dramatically expressed in renal proximal tubular epithelial cells within 1 week after inducing diabetes and gradually increased to week 8. MT-1/-2 expression seems to correlate with glucose level, but not with UAE, HbA1c, interstitial abnormalities. To our knowledge, this is the first report describing the localization and expression of MT-1/-2 in the diabetic kidney.

To elucidate the mechanism by which diabetes induces MT-1/-2 expression in proximal tubular epithelial cells, we investigated the effects of high glucose stimulation on mProx24, a murine renal proximal tubular epithelial cell line. We detected increased MT-1 mRNA and MT-1/-2 protein expression in the high glucose condition and found that high glucose-induced MT-1/-2 expression was suppressed by pretreatment with the antioxidant vitamin E. Vitamin E is well known to have high biological activity to protect cells from the propagation of free radical reactions [22, 23], thus we chose vitamin E in this study. These data suggest that ROS and oxidative stress, which are induced by high glucose, may be involved in the induction of MT-1/-2. Although

Table 1: Metabolic data at 1, 2, and 8 weeks after inducing diabetes.

	1 week	2 week	8 week
Body weight (g)			
Control	204 ± 6.3	241 ± 10.4	380 ± 13.3
Diabetic	198 ± 4.7	225 ± 11.5	248 ± 16.6*
Kidney weight (mg/g BW)			
Control	5.8 ± 0.4	5.6 ± 0.8	4.5 ± 0.7
Diabetic	5.9 ± 0.6	6.1 ± 1.0	6.7 ± 0.9*
UAE (μg/day)			
Control	110 ± 7.3	121 ± 8.1	137 ± 14.7
Diabetic	116 ± 5.7	125 ± 9.4	458 ± 24.5*
HbA1c (%)			
Control	3.7 ± 0.4	3.8 ± 0.6	3.8 ± 0.5
Diabetic	3.8 ± 0.3	4.3 ± 0.7	7.8 ± 0.9*

Data are means ± SEM; *$P < 0.05$ versus the control group. BW: body weight; UAE: urinary albumin excretion; HbA1c: hemoglobin A1c.

several studies have shown that MT protein expression is increased in the kidney of diabetic animals [16–18], the cellular distribution of MTs has not been addressed. Our data provide the first evidence for the expression profile of MT-1/-2 in the diabetic kidney. We speculate that MT-1/-2 is highly induced in proximal tubular epithelial cells in compensation for oxidative stress induced by high glucose.

Our study has potential limitations. First, we speculated that MT-1/-2 expression was upregulated by ROS, but further studies are needed to elucidate the underlying mechanisms. Although Zn is known to induce the gene and protein expression of MTs [24], this essential trace element is unlikely to be involved in our findings because the same chow was provided to the control and diabetic rats. In this study, we showed that high-glucose-stimulated MT-1/-2 expression was attenuated by vitamin E *in vitro*, but we have no data about diabetic rats treated by vitamin E. MT-1/-2 expression in the diabetic state may differ between cells and tissues, and the mechanisms by which other antioxidants regulate the expression of MT remain unclear. Further studies are needed to elucidate these issues. Second, it is still controversial whether site-specific induction of MT plays an important role in diabetic nephropathy. Podocyte-specific overexpression of MT reduced diabetic nephropathy in transgenic mice [25]. However, no studies have investigated whether MT expression in proximal tubular epithelial cells has a protective effect in diabetic animal models. Therefore, diabetes models using MT-knockout mice are needed to answer this question.

In conclusion, renal ROS, which are induced by diabetes, upregulate MT-1/-2 expression in proximal tubular epithelial cells of the kidney. Our results suggest that MT-1/-2 might be a novel therapeutic target to treat diabetic nephropathy.

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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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Decreasing serum uric acid levels might be associated with improving estimated glomerular filtration rate (eGFR) in Japanese men

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ABSTRACT

The link between changes in a subject's serum uric acid levels and his estimated glomerular filtration rate (eGFR) was evaluated in Japanese men. We used data for 108 Japanese men (45.3 ± 8.0 years) with a 1-year follow up. eGFR was defined by a new equation developed for Japan. eGFR was weakly correlated with serum uric acid levels ($r = -0.287$, $p = 0.0026$) at baseline. Subjects were given advice for dietary and lifestyle improvement. At the 1-year follow up, almost metabolic syndrome components were significantly improved. However, blood sugar and uric acid did not change and eGFR was significantly decreased. The changes in eGFR were weakly correlated with abdominal circumference ($r = -0.249$, $p = 0.0094$) and uric acid ($r = -0.340$, $p = 0.0003$). A decrease in serum uric acid levels may be associated with improving eGFR in Japanese men.

Keywords: Abdominal Circumference; Uric Acid; Estimated Glomerular Filtration Rate (eGFR); Metabolic Syndrome; Lifestyle Modification

1. INTRODUCTION

Chronic kidney disease (CKD) has become a public health challenge and is a common disorder [1]. For example, about 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 three 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 also showed that decreasing abdominal circumference in men and decreasing systolic blood pressure in women were associated with improving eGFR with lifestyle modification [5, 6]. In turn, there some reports according to the link between serum uric acid levels and CKD in foreign countries [7-12]. However, whether decreases in serum uric acid levels are beneficial for improving eGFR, and what affects this has on eGFR remain to be investigated in a longitudinal study in Japanese men.

In this study, we evaluate the link between changes in eGFR and changes in serum uric acid levels in Japanese men with a 1-year follow up.

2. SUBJECTS AND METHODS

2.1. Subjects

We used data for 108 Japanese men, aged 45.3 ± 8.0 years, who met the following criteria: 1) received a health check-up including special health guidance and a follow-up check-up 1-year later, 2) received anthropometric measurements, fasting blood examination including serum uric acid levels 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

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

	Baseline	Follow up	*p*
Number of Subjects	108		
Age	45.3 ± 8.0		
Height (cm)	168.9 ± 5.3		
Body weight (kg)	76.5 ± 11.5	74.9 ± 10.8	<0.0001
Body mass index (kg/m^2)	26.8 ± 3.5	26.2 ± 3.3	0.0001
Abdominal circumference (cm)	89.1 ± 9.9	86.9 ± 9.3	<0.0001
Systolic blood pressure (mmHg)	131.4 ± 14.5	123.6 ± 12.1	<0.0001
Diastolic blood pressure (mmHg)	82.4 ± 11.4	77.0 ± 8.9	<0.0001
Triglyceride (mg/dl)	158.1 ± 114.4	126.4 ± 83.0	0.0029
HDL cholesterol (mg/dl)	53.3 ± 14.5	55.6 ± 14.7	0.0260
Blood sugar (mg/dl)	103.4 ± 18.4	104.7 ± 29.6	0.4731
Uric acid (mg/dl)	6.1 ± 1.3	6.0 ± 1.3	0.3862
Cr (mg/dl)	0.80 ± 0.11	0.83 ± 13.3	0.0002
eGFR (ml/min/1.73 m^2)	85.0 ± 14.0	806. ± 13.3	<0.0001
		Mean ± SD	

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 amount of steps walked.

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

2.2. 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/[height]^2, in kg/m^2. Abdominal circumference was measured at the umbilical level in standing subjects after normal expiration [13].

2.3. 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.

2.4. 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.

2.5. Blood Sampling and Assays

We measured overnight fasting serum levels of createnine (Cr) (enzymatic method), uric acid, 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: eGFR (ml/min/1.73 m^2) = 194 × Cr$^{-1.094}$ × Age$^{-0.287}$ [3]. Reduced eGFR was defined as an eGFR < 60 ml/min/1.73 m^2. Serum uric acid levels were measured by the Uricase-Peroxidase method. The institutional normal range was 2.5 - 7.0 mg/dl.

2.6. 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 [13].

2.7. Statistical Analysis

Data are expressed as means ± standard deviation (SD). A statistical analysis was performed using a paired *t* test, χ^2 test and covariance analysis: $p < 0.05$ was considered to be statistically significant. Pearson' s correla-

tion coefficients were calculated and used to test the significance of the linear relationship among continuous variables; stepwise multiple regression analysis was also used.

3. RESULTS

The clinical parameters at the baseline and the 1-year follow up are summarized in **Table 1**. Anthropometric, body composition parameters and metabolic syndrome components, except blood sugar, were significantly improved with lifestyle modification after one year. However, serum uric acid levels did not change, and Cr was significantly increased and eGFR was significantly decreased. However, thirty five subjects was diagnosed as having metabolic syndrome at baseline and seventeen subjects was diagnosed as having metabolic syndrome, and subjects with metabolic syndrome were significantly reduced after one year ($p < 0.0001$). One subject was diagnosed with reduced eGFR at baseline and two subjects were diagnosed with reduced eGFR at the 1-year follow up. In addition, four subjects were identified as trace positive, two subjects were identified as positive (+) and one subject was identified as positive (2+) for proteinuria at baseline and five subjects were identified as trace positive, four subjects were identified as positive (+) and two subjects were identified 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**). To avoid the influence of age, we used age as a covariate and compared eGFR between men with and those without metabolic syndrome components using covariance analysis. There were no significant differences in eGFR between the groups with or without components of metabolic syndrome. In addition, eGFR in subjects with metabolic syndrome was similar to that in subjects without it, even after adjusting for age. Serum uric acid levels was negatively and weakly correlated with eGFR at baseline ($r = -0.287$, $p = 0.0026$) (**Figure 1**).

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 ($r = -0.249$, $p = 0.0094$) (**Table 3**). However, changes in eGFR were not significantly correlated with changes in other metabolic components. Changes in eGFR were negatively correlated with changes in serum uric acid levels ($r = -0.340$, $p = 0.0003$) (**Table 3**, **Figure 2**). We also used stepwise multiple regression analysis to evaluate the effect of changes in clinical parameters, *i.e.* age, abdominal circumference, systolic blood pressure, diastolic blood pressure, triglyceride, HDL cholesterol, blood sugar and serum uric acid levels on the change in eGFR, and found that only change in abdominal circumference and serum uric acid levels were significant [Change in eGFR = –5.296 – 0.330 (change in abdominal circumference) –3.259 (change in uric acid) , $r^2 = 0.149$, $p = 0.0002$].

4. DISCUSSION

Iseki *et al.* [14], Ninomiya T *et al.* [15] and Tanaka *et al.* [16] showed that metabolic syndrome, using the modified ATP III definition [17], was associated with CKD in the Japanese population. Compared with subjects with 0 or 1 component 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 [15]. In this study, thirty five

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

	Abdominal obesity (–)	Abdominal obesity (+)	*p*	*p* (Afrer adjusting for age)
Number of subjects	35	73		
eGFR (ml/min/1.73 m^2)	80.3 ± 14.5	87.2 ± 13.3	0.0168	0.6214
	Impaired glucose torelance (–)	Impaired glucose torelance (+)		
Number of subjects	84	24		
eGFR (ml/min/1.73 m^2)	83.9 ± 13.9	88.8 ± 13.8	0.1246	0.8832
	Hypertension (–)	Hypertension (+)		
Number of subjects	43	65		
eGFR (ml/min/1.73 m^2)	83.8 ± 15.5	85.8 ± 13.0	0.4688	0.8588
	Dyslipidemia (–)	Dyslipidemia (+)		
Number of subjects	60	48		
eGFR (ml/min/1.73 m^2)	84.0 ± 14.3	86.2 ± 13.6	0.4052	0.7367
	Metabolic syndrome (–)	Metabolic syndrome (+)		
Number of subjects	73	35		
eGFR (ml/min/1.73 m^2)	83.7 ± 14.2	87.6 ± 13.4	0.1852	0.3008

Mean ± SD

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

	r	*p*
Abdominal circumference (cm)	–0.249	**0.0094**
Systolic blood pressre (mmHg)	–0.101	0.2996
Diastolic blood pressure (mmHg)	0.025	0.7946
Triglyceride (mg/dl)	–0.050	0.6071
HDL cholesterol (mg/dl)	–0.044	0.6496
Blood sugar (mg/dl)	–0.037	0.7011
Uric acid (mg/dl)	–0.340	**0.0003**

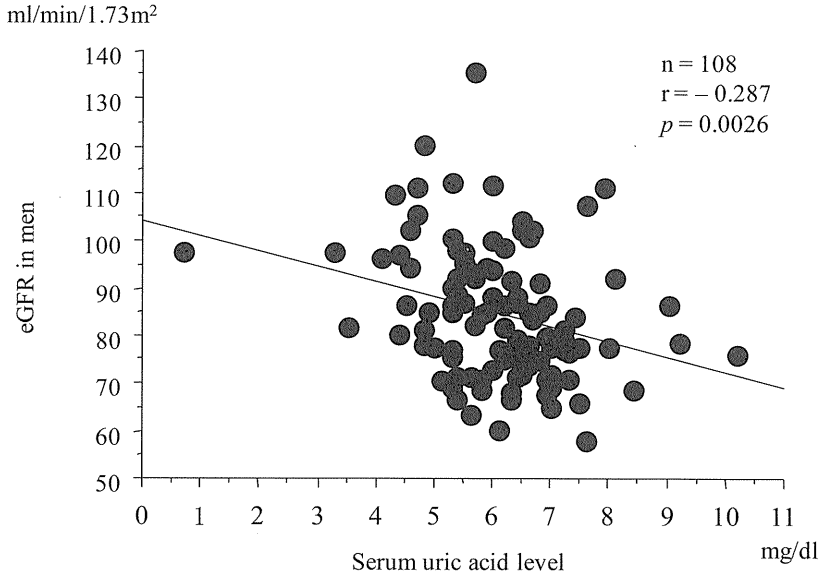

Figure 1. Simple correlation analysis between eGFR and serum uric acid levels at baseline.

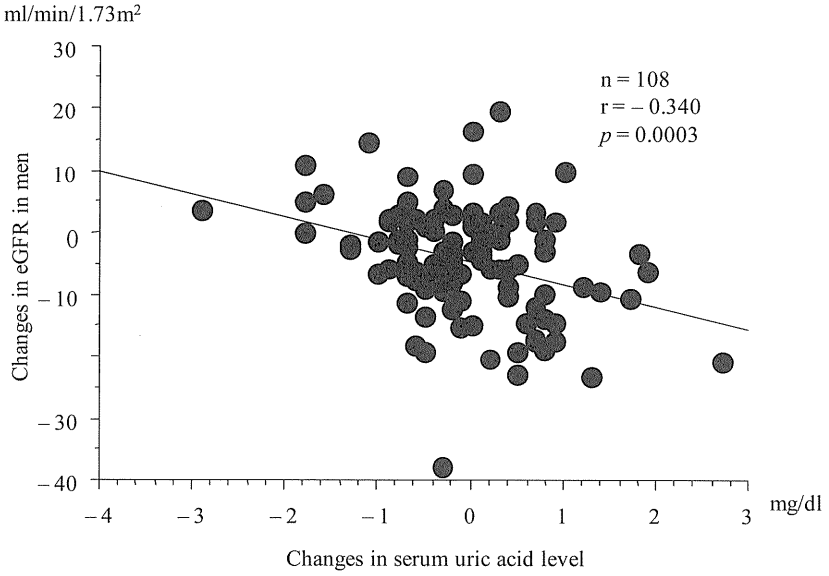

Figure 2. Simple correlation analysis between changes in eGFR and changes in serum uric acid levels at 1-year follow up.

